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Occurrence and Localization of Catechol Amines in the Human Brain

By

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Abstract

BERTLER, Å., *Occurrence and localization of catechol amines in the human brain.* Acta physiol. scand. 1961. *51.* 97—107. — The content of noradrenaline, dopamine and 5-hydroxytryptamine (5-HT) in various structures of the human brain has been investigated. Noradrenaline in rather high concentrations was found in the anterior and middle portion of the hypothalamus, and lower levels were recorded in structures of the mesencephalon and the floor of the 4th ventricle. Dopamine showed a very strict localization to structures which are involved in the extrapyramidal system, i. e. the basal ganglia and the substantia nigra suggesting that it may be involved in the function of this system. Relatively high concentrations of 5-HT were found in the hypothalamus, the medulla oblongata, the corpus striatum and the thalamus.

The data now available indicate that catechol amines are of importance for the normal brain function. A fact pointing in that direction is the localization of these amines to certain structures of the brain. Thus VOGT (1954) found that the brain regions containing the diencephalic, mesencephalic and bulbar representation of the sympathetic activities also were rich in noradrenaline and adrenaline. Studies on the third catechol amine known to occur in mammalian brain, i. e. dopamine (3-hydroxytyramine) revealed quite a different localization of this substance[1] (BERTLER and ROSENGREN 1959 a and b), high concentrations

[1] The results were first reported at Symposium on Catechol Amines, Bethesda, October 16—18, 1958 (CARLSSON 1959).

Table I. Distribution of noradrenaline and dopamine in the human brain

µg/g

Brain part	Number of estimations	Noradrenaline		Dopamine	
		Range	Mean	Range	Mean
Cortex from					
Frontal lobe	2	0.00–0.02	0.01	0.00–0.00	0.00
Anterior central gyrus	2	0.00–0.03	0.02		−0.01
Posterior central gyrus	2	0.02–0.03	0.03	−0.07–(−)0.05	−0.06
Occipital pole	1	—	0.00	—	0.06
Hippocampus + olfact. bulb.	1	—	0.03	—	−0.01
Septum pellucidum	1	—	0.03	—	0.04
Basal ganglia					
Caudate nucleus	10	0.00–0.08	0.04	1.50–4.67	3.12
Putamen	5	−0.01–0.05	0.02	3.02–7.72	5.27
Globus pallidus	4	0.00–0.08	0.05	0.11–0.55	0.32
Internal capsule (anterior limb)	1	—	0.00	—	0.42
Claustrum	3	0.00–0.07	0.02	0.16–0.67	0.44
Thalamus					
Anterior nuclei	1	—	0.02	—	0.07
Lateral nuclei	2	0.00–0.07	0.04	0.00–0.02	0.01
Medial nuclei	1	—	0.09	—	0.03
Hypothalamus					
Anterior parts	8	0.34–1.87	0.96	0.02–0.31	0.18
Intermediate part	7	0.24–1.80	1.19	0.03–0.24	0.14
Posterior part	8	0.03–1.01	0.31	0.00–0.39	0.22
Mesencephalon					
Superior colliculus	4	0.07–0.18	0.12	0.09–0.15	0.13
Inferior colliculus	4	0.06–0.33	0.15	0.00–0.15	0.10
Red nucleus	4	0.14–0.27	0.22	0.12–0.35	0.19
Substantia nigra	6	−0.06–0.08	0.04	0.25–0.70	0.40
Basis pedunculi	2	—	0.00	−0.03–0.00	−0.02
Rest	3	0.20–0.29	0.24	0.07–0.15	0.11
Pons	2	0.02–0.05	0.04	−0.01–0.01	0.00
Medulla oblongata					
Dorsal part, containing the floor of 4th ventricle	2	0.10–0.16	0.13	−0.01–0.00	−0.01
Ventral part	2	0.00–0.06	0.03	0.00–0.01	0.01
Cerebellum					
Dentate nucleus	1	—	0.01	—	0.08
Cortex	2	0.01–0.03	0.02	0.01–0.02	0.02
Pituitary gland	2	—	0.00	0.01–0.02	0.02

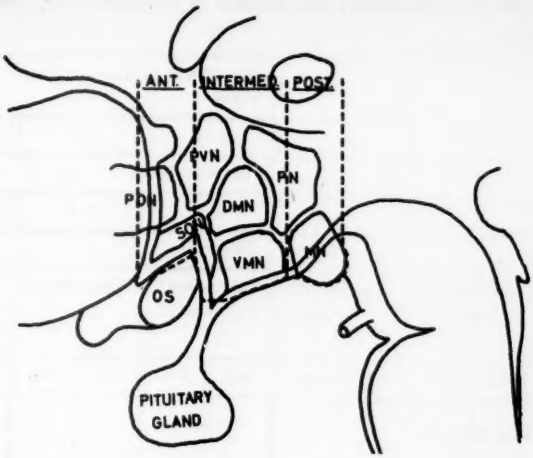

Fig. 1. Diagrammatic drawing of the hypothalamic nuclei. The broken lines indicate the boundaries between the subdivisions used in this investigation (see the text).

PON	Pre-optic nucleus
SON	Supra-optic nucleus
PVN	Paraventricular nucleus
DMN	Dorso-medial nucleus
VMN	Ventro-medial nucleus
PN	Posterior nucleus
MN	Mammillary nuclei
OS	Optic schiasm
ANT.	Anterior part
INTER-MED.	Intermediate part
POST.	Posterior part

being found in the corpus striatum of the cerebral hemispheres. Preliminary data from this laboratory have shown a similar distribution pattern of this amine in the human brain as in the brains of other mammalian species examined (BERTLER and ROSENGREN 1959 c). This finding was later confirmed by SANO et al. (1960) and by EHRINGER and HORNYKIEWICZ (1960).

In order to get further information about the function of the catechol amines, specially dopamine, in the central nervous system a more extensive knowledge of the localization of these amines within brain seemed of importance. The human brain appeared to be well suited for such an investigation, as the various structures can be more readily dissected out. In this paper a detailed account on the occurrence of noradrenaline and dopamine in the human brain is given. Some data on the presence of 5-hydroxytryptamine (5-HT) are also presented.

Material and Methods

The brain material was obtained from the autopsy room 1.5—5 hrs after death of the patient. Each piece of tissue was immediately cut into small slices and put into a glass tube containing 0.4 N perchloric acid. Estimation of catechol amines was performed according to the methods developed in this laboratory (BERTLER, CARLSSON and ROSENGREN 1958, CARLSSON and WALDECK 1958). In order to check the identity of the substances determined, paper chromatography has been performed in a few cases. The activation and fluorescence spectra of the fluorophores obtained from the brain material were always compared to those of synthetical noradrenaline or dopamine. The method used for estimation of 5-HT has been briefly outlined by BERTLER and ROSENGREN (1959 c). A more detailed account of this method will be published elsewhere (BERTLER 1960).

Table II. Noradrenaline and dopamine in 10 human brains, from which four or more structures μg/g

	Age of the patient Years							
	75		52		73		83	
	Hrs after death							
	2		2		2		2	
	Diagnosis							
	Pulmonary embolism		Heart failure		Mammary cancer		Cerebral arterio-sclerosis	
	NA	DA	NA	DA	NA	DA	NA	DA
Basal ganglia								
Caudate nucleus	0.00	2.80	0.06	3.75			0.05	2.45
Putamen								
Globus pallidus								
Internal capsule								
Claustrum								
Hypothalamus								
Anterior part	1.13	0.31	0.78	0.10			0.49	0.12
Intermed. part	1.44	0.13	1.80	0.24			0.24	0.04
Posterior part	0.34	0.28	1.01	0.30			0.17	0.00
Mesencephalon								
Superior colliculus			0.18	0.09	0.16	0.09		
Inferior colliculus			0.33	0.12	0.16	0.06		
Red nucleus			0.22	0.10	0.24	0.19		
Substantia nigra			0.08	0.51	0.07	0.40		
Basis pedunculi					0.00	0.03		
Rest			0.29	0.15	0.23	0.15		

Results

The concentrations of noradrenaline and dopamine in different parts of the human brain are seen in Table I. Most of the dopamine present in the brain was found to be localized to some nuclei of the basal ganglia of the cerebral hemispheres. Thus the caudate nucleus and the putamen contained 3 and 5 μg per g, respectively. The dopamine contents of the globus pallidus and the claustrum were about 10 times smaller. In all the structures of the basal ganglia the concentration of noradrenaline was 0.05 μg per g or less. In the rest of the cerebral hemispheres the concentrations of catechol amines were very small.

have been taken for estimation

Age of the patient Years											
78		76		60		79		87		74	
Hrs after death											
2.5		3		4		4		4		5	
Diagnosis											
Myocardial infarction		Mammary cancer		Leukemia		Circulatory failure		Oesophag. cancer		Myocardial infarction	
NA	DA	NA	DA	NA	DA	NA	DA	NA	DA	NA	DA
		0.03	2.80	0.03	4.67					0.03	3.65
		0.02	3.51	0.04	5.54					0.02	3.02
		0.00	0.22	0.00	0.55	0.07	0.11	0.04	0.39		
										0.00	0.42
				0.00	0.67						
				0.59	0.20	0.34	0.21	0.48	0.10		
						0.52	0.21	0.64	0.21		
				0.09	0.11	0.03	0.12	0.25	0.30		
0.06	0.15	0.12	0.15								
0.12	0.12	0.07	0.00								
0.14	0.12									0.14	0.26
0.02	0.44					0.03	0.25			0.03	0.60
0.00	0.00										
0.20	0.07										

Noradrenaline was found in a fairly high amount in the hypothalamus, where relatively small amounts of dopamine, 0.2 μg per g, could be detected. Within that structure most of the noradrenaline was recorded in the anterior and intermediate parts. In the former part the preoptic nucleus, most of the supraoptic nucleus and a piece of the paraventricular nucleus were included (Fig. 1), while the intermediate part contained the rest of the paraventricular nucleus, the dorsomedial nucleus and the ventromedial nucleus and part of the posterior nucleus. The posterior part of the hypothalamus was principally made up of the rest of the posterior nucleus and the mammillary nuclei.

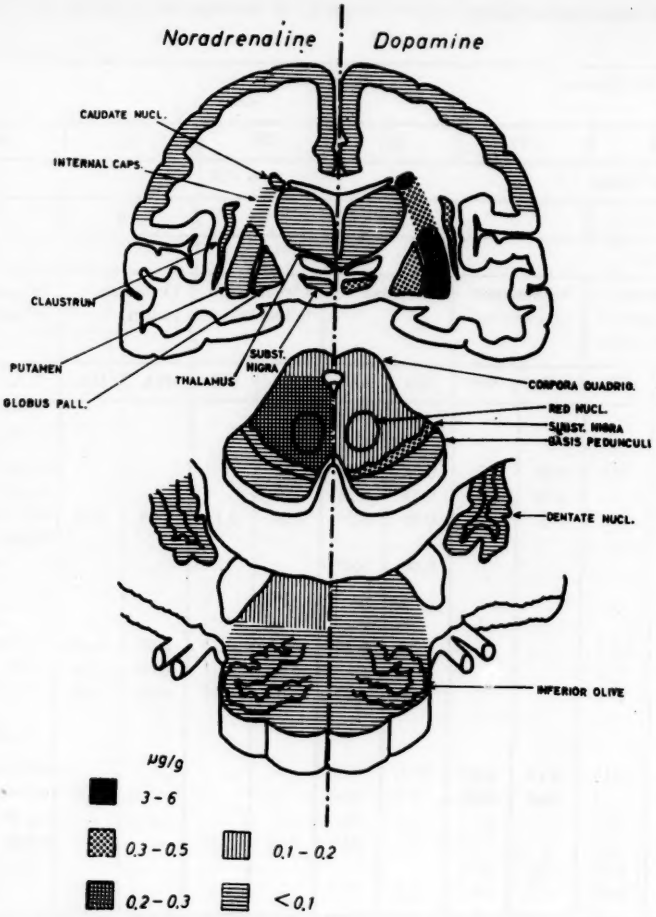

Fig. 2. Noradrenaline and dopamine in the human brain.

In the midbrain noradrenaline, in a concentration of more than 0.2 μg per g was found in the red nucleus. A sample containing, among other structures, the mesencephalic part of the reticular formation and the mesencephalic nuclei of the eye, had about the same concentration of noradrenaline. Dopamine was principally concentrated to the substantia nigra where noradrenaline was nearly absent. Significantly lower concentrations of dopamine were recorded in the other parts of the mesencephalon.

Table III.

1. Brain from a 5 months old foetus, taken 4 hrs after death. The sample called "central part" containing the basal ganglia, the thalamus, the hypothalamus and some adjacent structures.

μg/g

	Noradrenaline	Dopamine
Central part	0.11	0.07
Mesencephalon + pons	0.14	0.04

2. Brain from an 8 months old foetus, taken 3.5 hrs after death. Diagnosis: Heart failure.

μg/g

	Noradrenaline	Dopamine
Caudate nucleus	0.07	1.27
Putamen	0.08	2.11
Globus pallidus	0.07	0.31
Hypothalamus	0.24	0.00
Corpora quadrigemina	0.14	0.12
Basis pedunculi	0.00	0.15
The midbrain except the corpora quadrigemina and the basis pedunculi	0.20	0.21
Floor of the 4th ventricle	0.28	0.06

In the medulla oblongata some noradrenaline was found in the part containing the roof of the 4th ventricle. In this part the nuclei of several cranial nerves and another part of the reticular formation are found. No dopamine could be detected here. In the cerebellum no significant amounts of catechol amines could be detected. The localization of the two catechol amines in brain is schematically shown in Fig. 2.

Two foetal brains have been examined, and the results are given in Table III. The first of them was taken from a 5-month-old foetus. In this case the basal ganglia, the thalamus, the hypothalamus and some adjacent structures were analyzed together. Small amounts of noradrenaline could be detected in this sample but the dopamine level was proportionally still lower. The other brain examined was taken from an 8-month-old foetus, where a more detailed dissection could be performed. In this case some noradrenaline was found in the hypothalamus, the midbrain and the floor of the 4th ventricle. Dopamine in a concentration of about 1 and 2 μg was found in the caudate nucleus and the

Table IV. Distribution of 5-hydroxytryptamine in the human brain

μg/g

	Age of the patient				Mean
	52	78	73	43	
	Hrs after death				
	2	2	1.5	2	
	Diagnosis				
	Heart failure	Myo-cardial infarction	Mammary cancer	Diabetes	
Cortex					
Frontal lobe		0.04			0.04
Occipital lobe		0.06			0.06
Hippocampus + olfactory bulb			0.11		0.11
Septum pellucidum		0.03			0.03
Basal ganglia					
Caudate nucleus	0.26	0.22	0.37	0.24	0.27
Putamen	0.22	0.23			0.23
Globus pallidus	0.08				0.08
Thalamus					
Medial nuclei	0.13		0.35		0.24
Lateral nuclei	0.13		0.30		0.22
Hypothalamus					
Anterior part			0.31	0.38	0.35
Intermediate part			0.32	0.64	0.48
Posterior part			0.29	0.30	0.30
Pons		0.19			0.19
Medulla oblongata					
Dorsal part containing the floor of 4th ventricle			0.50		0.50
Ventral rest			0.31		0.31

putamen respectively. Small but significant amounts were recorded in the globus pallidus and structures of the mesencephalon.

Some analyses on 5-hydroxytryptamine have been performed on samples from adult human brains (Table IV). This amine seemed to be more evenly distributed than the two catechol amines, concentrations between 0.2—0.5 μg per g being found in most of the tissues examined.

Discussion

Noradrenaline in the human brain is localized in a similar way as in the brains of other mammalian species, which have been investigated earlier (VOGT 1954, BERTLER and ROSENGREN 1959 b). Thus the highest concentrations of this amine were found in the parts which generally are considered to contain the central representation of the sympathetic activity. This includes both the central representation of the peripheral adrenergic nerves and that of the adrenal medulla. A neurogenically induced release of catechol amines from the adrenal medulla is often used as a criterion of activation of sympathetic centers. It is known, however, that the activity of the centre of peripheral adrenergic nerves and that of the adrenal medulla may vary independently of each other. Insulin hypoglycemia, for example, activates the adrenal medulla, *via* its centers in the brain without an appreciable activation of the adrenergic nervous system. On the contrary, during muscular work the adrenergic nervous system is activated without much engagement of the adrenal medulla (VENDSALU 1960). The results of this investigation indicate that the hypothalamic noradrenaline is present at higher concentration in the anterior and intermediate parts. It is from this fact, however, impossible to conclude that these parts of the hypothalamus are the site of "sympathetic centres", and one can put forward the question, if noradrenaline always should be found at centers with "sympathetic activities". It may as well be a transmitter in quite a different system at a high synaptic level, just as acetylcholine is the transmitter between the pre- and postganglionic fibers of the adrenergic nervous system.

Dopamine, on the other hand, is strongly localized to structures which are involved in the motor functions of the organism, i. e. the basal ganglia and structures in close relation to these nuclei, such as the substantia nigra. The distribution within the basal ganglia is interesting, too, as most of this amine was found in the phylogenetically youngest parts of this brain structure.

In a recent investigation SANO et al. (1960) has found larger amounts of dopamine in the caudate nucleus and the putamen than we have observed in this investigation. They also reported the occurrence of considerable amounts of the amine in structures with no or very little connection to the basal ganglia. This discrepancy is probably due to methodological differences. As the condensation reaction with ethylene diamine is not specific for catechol amines it is possible that some other material has been estimated, too.

From the data now available it may be concluded that noradrenaline in the brain can be of importance for higher centres of the adrenergic nervous system or of the adrenal medulla or both but it may be involved in other cerebral functions as well.

Dopamine is concentrated to structures about the function of which very little is known, except that they in some way are involved in the motor functions of the organism. Results from surgical and pathological studies indicate that

the activity of the globus pallidus is i. a. influenced by the striatum and the substantia nigra. The globus pallidus is said to be the older and dynamic central nucleus in the basal ganglia. When pathological changes occur in these regions it may result in involuntary movements or muscular rigidity (see MARTIN 1959). Dopamine may play a role for the function of these nuclei, and this assumption is supported by the fact that reserpine, which depletes the brain of dopamine (CARLSSON et al. 1958), can produce side effects resembling those seen in patients with lesions in the basal ganglia or the substantia nigra. Further, reserpine has in some cases a favourable effect in patients suffering from Huntington's chorea (GRUNG and SUNDBY 1947, BRANDRUP 1960), where the pathological changes specially are localized to the putamen. In cases of Morbus Parkinson and postencephalitic Parkinsonism, a significant decrease of the dopamine content in the neostriatum may be seen (EHRINGER and HORNYKIEWICZ 1960).

Catechol amines in foetal brains

The preliminary results from foetal brains indicate, that noradrenaline and dopamine were present in a brain from a 5-months-old foetus. The concentration of dopamine was however very low. In a three months older foetus the dopamine concentration had nearly reached the levels found in the adult human brain. The noradrenaline concentration seemed to be increased, too, but the figures indicate, that dopamine had increased at a higher rate. It is possible, that this amine not only is phylogenetically but also ontogenetically a young substance in the brain.

Occurrence of 5-hydroxytryptamine

Results from the few investigations made on 5-HT in the human brain showed low figures, possibly due to a very rapid break down of the amine. The distribution, however, seemed to be similar to that found in dog brain by BOGDANSKI, WEISSBACH and UDENFRIEND (1957), with the highest concentrations in the hypothalamus, but appreciable amounts also being present in the medulla oblongata, the striatum and the thalamus (Table IV). The figure obtained by these authors from the hypothalamus of a human brain is of about the same order of magnitude as that presented in this investigation. The thalamus is the only structure examined with relatively high 5-HT-concentration and very low catechol amine level.

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Brief Cyclic Variations in Some Sexual Functions of the Male Rabbit

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Abstract

G. DEGERMAN and J. E. KIHLSTRÖM. *Brief cyclic variations in some sexual functions of the male rabbit.* Acta physiol. scand. 1961. *51.* 108—115. — It has been demonstrated by means of two different statistical methods that at least two sexual functions of male rabbits, the volumes of daily ejaculates and the intensity of the sexual drive, are subjected to cyclic variations. The length of the cycle varies individually from 3 to 6 days, and is in some individuals superimposed by a longer period.

In contrast to the well-known and thoroughly studied estrus cycle of female animals, related phenomena are practically unknown in males. There exist only a few reports indicating the occurrence of sexual periodicity in males, one dealing with the sperm count (DOGGETT 1956), another with the volumes of the ejaculates (KIHLSTRÖM 1958). These observations are of interest from a theoretical as well as from a practical point of view, and justify a more systematical investigation, including a study of possibly existing periodic variations in sexual processes not connected with the genital secretions. In addition to the volumes of the ejaculates we therefore have chosen to study the sexual drive of the animals.

Material and methods

From Oct. 8, 1959 to Jan. 22, 1960 semen was daily collected from seven male rabbits (Swedish White Landrace), ranging in age from 10 to 31 months. The environment of the animals was maintained as constant as possible. They were thus given the same sort of food once a day at the same hour. Except for a few days at the beginning of the experiments the bucks were kept in separate cages which were not moved. The collecting of semen was performed at 1 p. m., and the animals always yielded their ejaculates in the same sequence, thus avoiding varying visual stimulation.

One ejaculate per day was collected from each rabbit by means of an artificial vagina, and the semen transferred into small test tubes. The volumes of these tubes up to a mark in the glass wall were known with great accuracy. By adding distilled water, or in some cases a physiological salt solution, from a burette up to this mark the volume obtained in the tube could be determined with a high degree of precision. However, a part of the ejaculate may remain in the vagina, thus decreasing the exactness of the determinations. The error was therefore estimated by measuring the volumes recovered in the test tube after having placed precisely known volumes of semen in the vagina. This gave a standard deviation of 0.05 between paired determinations.

Six per cent of the ejaculates contained a gelatinous coagulum which was removed before the determination of the volume. About three per cent of the ejaculates were contaminated with urine which is easily observed by its colour and odour. Such ejaculates, restricted mainly to two individuals, have been excluded.

Sometimes the animals showed the ejaculatory behaviour without delivering any seminal fluid. This being the case the volumes were noted as zero. Each day the artificial vagina was presented to the animal during a maximum time of 5 min. If no ejaculate was obtained within this time the vagina was withdrawn, and the buck looked upon as refusing to copulate. In all about 700 ejaculates have been studied, the mean volume being 0.38 ml.

In order to estimate the intensity of the sexual drive the time elapsing from the moment, when the vagina was presented to the animal up to the moment, when the animal had mounted the skin and begun the pelvic thrusts was measured by means of a stopwatch. This space of time varied widely, the average being 16.9 sec.

We have looked upon the pelvic thrusts as being a better terminal point of the time measured than the ejaculation (cf. WALTON 1949). Of course these two phenomena are both dependent upon exogenous as well as endogenous factors. The moment of ejaculation is thus intimately dependent upon the skilfulness of the person operating the vagina, and is evoked when the penis comes into contact with the warm rubber tube of the vagina. The beginning of the pelvic thrusts on the other hand is independent of the position of the vagina. Nevertheless many exogenous factors may influence the time measured. However, it is very improbable that these exogenous factors should vary rhythmically. Besides it seems likely that the sexual drive is the most important factor in evoking the pelvic thrusts. Consequently we may look upon this time, t, as being approximately inversely proportional to the libido of the animal. However, in diagrams as well as in statistical treatment, it would be preferable to use an expression, in which the figure mounts and falls with the intensity of the sexual drive. In order to get such a measure of libido we have used the value $10 \times t^{-1}$, expressed in an arbitrary unit, called libido unit. It must be pointed out that this measure presupposes that the vagina is presented to the animals under standard conditions. This could not be performed with rabbit no. 2 because this animal often needed the presence of a doe in order to be sexually excited. No measures of libido could consequently be obtained.

The statistical treatment of the data obtained has been performed in two different ways. Firstly we have used a test for randomness in series of numerical observations, elaborated by KERMACK and MC KENDRICK (1937). In this test figures greater than either of their immediate neighbours in the series are defined as maximum numbers. The interval between two such maximum numbers, expressed in time units, in our case days, is called a gap, both maximum numbers being included within the gap. In series of random numbers the theoretical distribution of gaps of different sizes has been calculated by KERMACK and MC KENDRICK (1937). By means of χ^2-analysis this theoretical distribution is then compared with the distribution found in the material to be tested. The method also involves a calculation of the error of the mean size of the gaps, which gives the

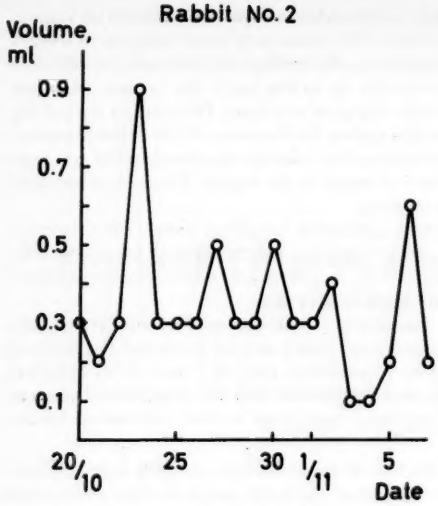

Fig. 1. Daily variations in the volumes of the ejaculates.

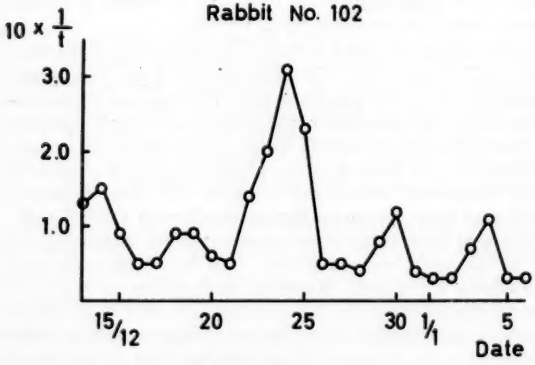

Fig. 2. Daily variations in the sexual drive. t = time in seconds from the presentation of the artificial vagina up to the beginning of the pelvic thrusts.

quotient $\frac{s^2}{\sigma^2}$ as an expression of the difference between observed and theoretical populations of gaps.

Using this method the only relevant fact is the interval between maximum numbers, the absolute magnitude of these numbers being irrelevant. In practice this means that very small and only occasionally occurring maxima are of the same importance in the calculations as greater and more pronounced maximum values. This in its turn gives a surplus of small gaps.

The other method is known as periodogram analysis which includes harmonic analysis of the periods obtained (cf. BRUNT 1917). In contrast to the method of KERMACK and MC KENDRICK (1937) the periodogram analysis is not influenced by small and only occasionally occurring peaks. Besides it permits us to calculate the length of the true period and to decide, whether only one period exists or we have more than one period superimposed upon each other.

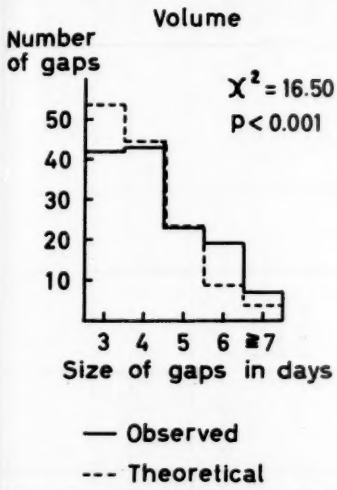

Fig. 3. The observed distribution of the sizes of the gaps between maximum volumes of the ejaculates, compared with the theoretical distribution to be found in a series of random numbers.

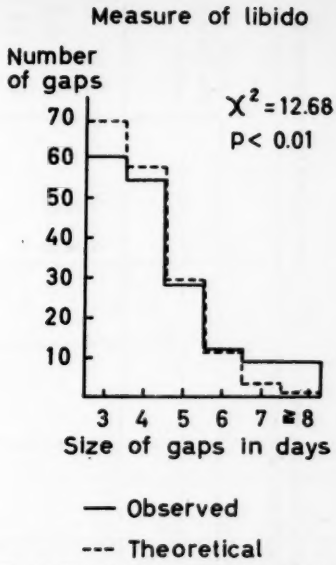

Fig. 4. The observed distribution of the sizes of the gaps between maximum measures of libido compared with the theoretical distribution to be found in a series of random numbers.

It is also possible to estimate the probability that the cyclic variations found are statistically different from random variations. The periodogram analysis has been employed for studying possibly existing cyclic variations up to a length of 14 days.

Table I. Test for randomness. Comparisons between distributions of gaps

	χ^2	n	P	$\frac{s^2}{\sigma^2}$	P
Volumes of the ejaculates	16.50	4	< 0.001	10.40	< 0.005
Measure of libido	12.68	4	< 0.01	18.57	< 0.0005

Table II. Mean characteristics of the periods found

	Volumes of the ejaculates	Libido
Gap between two maximum numbers (both maxima included)	4.31 days	4.36 days
Run down (maximum and minumum included)	2.61 days	2.60 days
Run up (minimum and maximum included)	2.70 days	2.74 days
Mean difference between maximum and minimum numbers	0.4 ml	1.3 libido units

Table III. Periodogram analysis

a = the amplitudes of the harmonic periods; k = the quotient between the square of amplitude examined and the mean square of the check amplitudes; p = the probability that the results are *not* due to a real periodic cause. Significant values in italics.

A. Volumes

Rabbit No.	Trial periods in days								True periods in days
	3	4	5	6	7	8	9	10	
2	a = 7.7 k = 6.0 *p = 0.0025*	a = 12.0 k = 14.8 *p < 8 × 10⁻⁷*	a = 6.0 k = 3.7 *p < 0.05*					a = 4.4 k = 2.0 p = 0.14	3.7
28	a = 22.16 k = 9.1 *p < 3.4 × 10⁻⁴*			a = 14.0 k = 3.6 *p < 0.05*					3.4 6.4
39	a = 1.4 k = 0.13 p < 0.9			a = 10.6 k = 7.5 *p < 0.0025*					5.8
44	a = 8.5 k = 3.8 *p < 0.05*	a = 8.3 k = 3.6 *p < 0.05*	a = 8.2 k = 3.4 *p < 0.05*			a = 8.7 k = 3.9 *p < 0.05*			3.5 8.5
47	a = 12.1 k = 5.0 *p = 0.007*		a = 11.2 k = 4.3 *p < 0.018*						3.4 5.5
102	a = 8.1 k = 8.1 *p < 3.4 × 10⁻⁴*	a = 17.3 k = 36.6 *p < 9.4 × 10⁻¹⁴*	a = 11.4 k = 16.2 *p < 1.1 × 10⁻⁷*						4.4
103	a = 15 k = 7.5 *p < 0.0025*		a = 6.8 k = 1.6 p < 0.22						3.3

B. Libido

Rabbit No.	Trial periods in days: 3	4	5	6	7	8	9	10	True periods in days
2	No figures obtained								
28	a = 39.1 k = 7.6 $p < 0.0025$	a = 42.5 k = 9.0 $p < 3.4 \times 10^{-4}$		a = 36.5 k = 6.6 $p < 0.0025$					3.5 6.3
39	a = 36 k = 2.0 p = 0.14	a = 61 k = 5.8 $p < 0.007$			a = 36.5 k = 2.1 p < 0.14				3.4
44	a = 7.8 k = 0.6 p < 0.5		a = 20.8 k = 4.5 $p < 0.01$						5.3
47	a = 45 k = 6.2 $p < 0.0025$	a = 57 k = 10.0 $p < 4.5 \times 10^{-5}$						a = 53 k = 8.7 $p < 3.4 \times 10^{-4}$	3.6 10.3
102	a = 41 k = 6.8 $p < 0.0025$	a = 9.9 k = 0.4 p < 0.67							3.2
103	a = 7.2 k = 0.2 p = 0.82	a = 12.2 k = 0.5 p < 0.67	a = 44.5 k = 7.1 $k < 0.0025$	a = 24.7 k = 2.2 p < 0.14					5.3

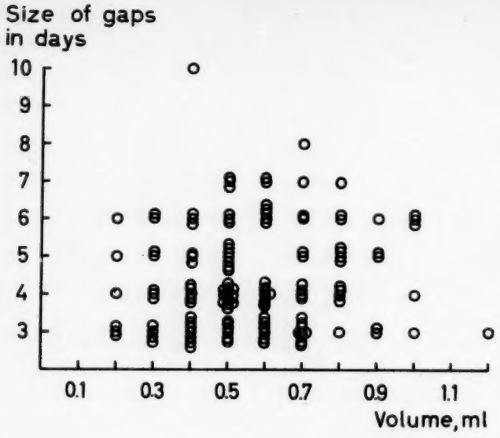

Fig. 5. The sizes of the gaps between maximum volumes plotted against the first maximum volume within the gap in question.

Results

The values obtained have been plotted in diagrams, as in Fig. 1 and 2. When using the test for randomness, devised by KERMACK and MC KENDRICK (1937), it was necessary to pool the values from all rabbits in order to get a sufficiently large statistical material. The comparisons by means of χ^2-analysis between the distributions of gaps observed and of those calculated are given in Fig. 3 and 4. As seen from these diagrams we must look upon the series of numbers obtained as not being random. In other words, we have strong indications of a really existing periodicity in the volumes of the ejaculates as well as in the intensity of the sexual drive. The same results are obtained by calculating the quotient $\frac{s^2}{\sigma^2}$ (See Table I).

The mean characteristics of the periods found by this method are given in Table II. As seen from the table the two properties studied show mean cycles nearly identical in length and form.

When using the periodogram analysis figures obtained from different individuals could be treated separately. The results are summarized in Table III. Beginning with the volumes of the ejaculates this table reveals that all animals show a primary cycle with a statistically probable length varying from 3.3 to 5.8 days. Besides, in 3 out of 7 rabbits we find a secondary cycle superimposed upon the shorter one. This longer cycle varies in length from 5.5 to 8.5 days.

As mentioned earlier it was impossible to estimate the libido of rabbit no. 2. The remaining six rabbits all show periodic variations with a primary cycle varying individually in length from 3.2 to 5.3 days (See Table III). In two

individuals this shorter period is superimposed by a longer one, the length of which is 6.3 and 10.3 days, respectively.

In one animal, rabbit no. 28, we find the two properties studied to have approximately equal probable lengths of the cycles. Nevertheless, the cycles are not synchronous.

Discussion

As to the causes of the periodicities found it is possible that an unusually big ejaculate may be followed by a strikingly long interval before the sexual glands have produced enough secretions to yield another big ejaculate. If this is the case we should expect to find a positive correlation between the maximum volumes and the intervals following up to the next maximum volume. However, no such correlation could be revealed (See Fig. 5).

The periodical variations may also depend upon a varying irritability of the nerves supplying the genital organs, or the genital organs themselves may vary in their response to the sexual hormones. However, the parallelism in length between the periodical variations found by the present authors and those demonstrated by DOGGETT (1956) strengthens the impression that these variations are different expressions of cyclic variations in the hormonal status, most likely in the circulating amount of androgen hormones. Work is in progress with a view to elucidate these problems.

In most handbooks the length of the estrus cycle of female rabbits is said to be 7 to 10 days (ASDELL 1946, PARKES 1956). However, HAMILTON (1951) has found a female cycle of 4—6 days, which is in agreement with the length of the male sexual cycle found in the present work.

We are indebted to the Head of the Institute, Professor P. E. LINDAHL, for valuable discussions. Financial support from Lars Hierta Memorial Foundation is gratefully acknowledged.

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Hemodynamic Studies with Differential Pressure Technique

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Abstract

PORJÉ, I. G. and B. RUDEWALD. *Hemodynamic studies with differential pressure technique.* Acta physiol. scand. 1961. *51.* 116—135. — A theoretical analysis of the fluid motion in a circular elastic tube is presented. The formulae for computation of pulsatile flow and other hydrodynamic data are discussed. Apparatus and technique for recording differential pressure are described. Results of studies of the hydrodynamics in a model circulation system show that the theory is valid. For kinematic viscosities ranging from 1—9 cst the differential pressure is in the main unaltered. Original differential pressure records from the ascending aorta in cases with normal aortic valves are presented and their features described. Blood velocity curves computed from such curves are presented. To illustrate the use of the method the effect of the Valsalva manoeuvre and a nitrite (Etrynit, Bofors) upon the aortic blood motion are demonstrated.

Heart catheterization has opened possibilities for quantitative determination of the cardiac output per minute and the stroke volume according to the Fick or the Hamilton principles. Thereby valuable clinical and physiological data about the circulation in intact man is obtained. These methods give mean values for the flow during a certain interval of time but no information about the dynamics of a particular heart stroke. For the direct measurement of the flow there are several types of flowmeters but as yet none can be used safely to measure the instantaneous aortic blood flow in intact man. Our work has been devoted to the development of a method for the study of the dynamics of the aortic blood motion, based upon differential-pressure curves recorded from the aorta.

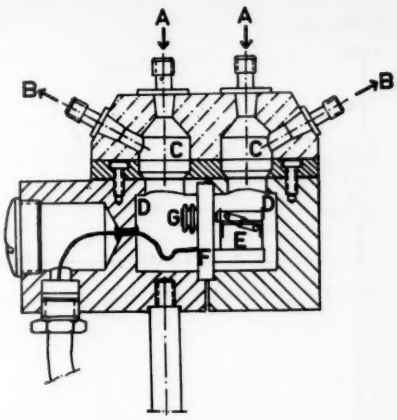


Fig. 1. Diagram of the pressure transducer. For description, see text.

The theoretical and experimental problems met with in the hemodynamics of the arterial circulation are extremely intricate. These problems have recently been reviewed by McDONALD and TAYLOR (1959). The theoretical works by WOMERSLEY have particularly contributed to the understanding of the dynamics of the pulsatile flow. In earlier works (1957 a, b) we have reported on the dynamics in the ascending aorta in human beings. FRY *et al.* (1956) reported on studies of the blood flow in the dog's aorta with a technique similar to ours. This group has continued their work and also investigated the aortic blood flow in human beings.

The motion of a Newtonian fluid in an elastic tube can be described by partial differential equations. In their general form these equations cannot yet be solved in an explicit form. By suitable hypotheses, however, about the deformations of the tube and the effect of the viscosity we can solve the equations. Our aim is to achieve formulae relating flow or velocity to some physical magnitudes that can be measured in vivo. It is obvious that the non-Newtonian behaviour of the blood, the visco-elastic properties of the vessels, and their biological activity will be factors that limit the theoretical approach.

For the theoretical part of this study the reader is referred to the addendum.

Apparatus

As the reliability of the pressure difference curve is very important for our calculations, the demands on the recording system must be high. Our first Δp-curves from human beings were obtained with two separate catheters attached to a particular differential pressure transducer. As accelerations and bendings of the catheters may cause distortion of the Δp-curve, double lumina catheters should be preferred. As no suitable catheters were available for our purpose, different types were designed in cooperation with

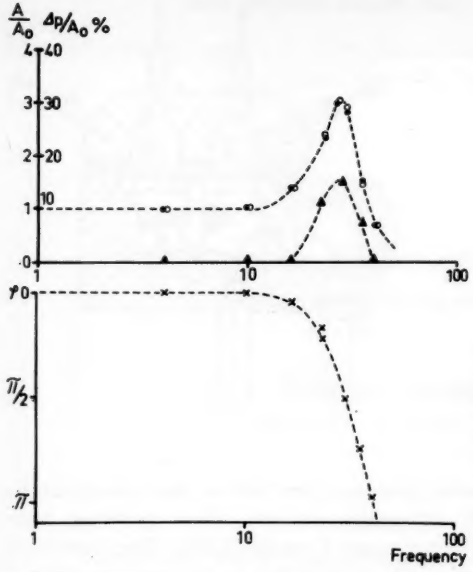


Fig. 2. Dynamic properties of the differential pressure transducer and 100 cm long double lumina catheter. Upper diagram shows the amplitude response (A/A_0) as a function of frequency for both sides of the pressure transducer (• and ○). Solid triangles (▲) represent the dynamic imbalance of the system. Lower diagram shows the phase lag for different frequencies.

Svenska Metallverken and U. S. Catheter & Instr. Corp. The Ödman-Ledin material is used in the former catheter. The outer diameter of the catheter should be as small as possible to render it suitable for percutaneous insertion in the vascular system. These demands necessitate an improved performance of the differential pressure transducer.

The type used here is constructed in collaboration with *Ing. A. Söderholm and Ing. P. Engström* at the Division of Aeronautics at the Royal Institute of Technology, Stockholm. The following technical description is given by them.

The pressure transducer (Fig. 1) consists of two separate outer plexiglass chambers (C) for the attachment of the catheter (A) and airing (B). The outer chambers are separated from two inner oil-filled chambers (D) by flat elastic membranes. The transducing member (E) consists of an unbonded strain gauge element built up of a parallelogram system and filament coils with a length of less than 1/2 inch. The element is fastened to a partition (F) between the chambers. Round a circular hole in the partition a bellow (G) is fastened to which the strain gauge element is connected. The bellows are acted upon by both liquid pressures and transfer a force to the strain gauge element proportional to the difference between the pressures. The filament coils are prestrained. To avoid the cracking of filaments, spacers are inserted between them (Swedish patent no. 159812) in such a way that the forces upon the spacers are balanced (see ref.). By this method the prestrain is found to be very uniform resulting in good zero stability *i. e.* negligible fluctuation of the zero point. The moving mass of the transducer element is kept small and in combination with a high spring constant the natural frequency of the strain gauge element is high. The volume displacement is about 0.3×10^{-6} mm³/mm of mercury.

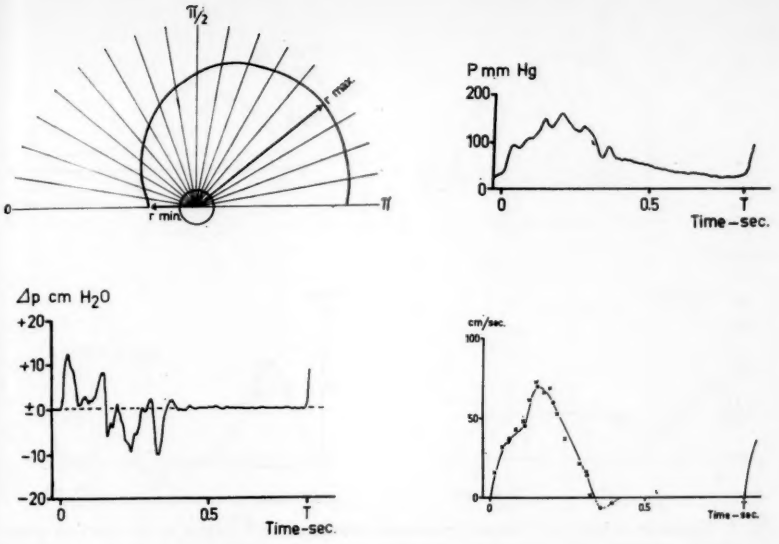


Fig. 3. Upper left diagram shows the excentric that is driven by a motor at a constant r.p.m. When the excentric turns from r_{min} to r_{max} liquid is ejected from the pump. During the rest of the revolution there is zero flow. Redrawn differential pressure curve (in cm H_2O) is shown below. Computed values for fluid velocity (in cm/sec.) are indicated by heavy dots (•). Corresponding values calculated from the profile of the excentric are plotted for comparison (×). The "stroke volume" is 66 cm^3. Computed value 64 ± 4 cm^3.

Our present system of registering with a double lumina catheter, length 100 cm, and outer diameter corresponding to USCI no. 6,5 has the frequency response shown in Fig. 2. There is a flat amplitude response for frequencies lower than about 14 hz and the phase-lag is negligible for the same range of frequencies. It is observed that for higher frequencies there is a resonance peak with a maximum at about 26 hz. This means that signals containing frequencies within this range will be distorted. In the frequency spectrum of the aortic pressure curve the amplitude of harmonies higher than the 7:th is only a very small percentage of the fundamental, (ref. no. 7) and thus must be very low. Therefore the dynamic properties of the recording system will be sufficient for the measurements referred to here. It is however, desirable to improve the high frequency response as will be done in a new apparatus.

The catheter is provided with lateral openings and the tip is filled with a heavy metal. The increased mass renders the catheter tip less sensitive to small high frequency vibrations. The pressure difference has been recorded in a circulation model and human beings with type of catheter. A simple test of the performance of the recording system can be carried out by shifting the catheter endings to opposite sides of the pressure transducer. This manipulation will give a curve that is the original one with a negative sign. As even very small air bubbles will cause asymmetry they are easily detectable by this test.

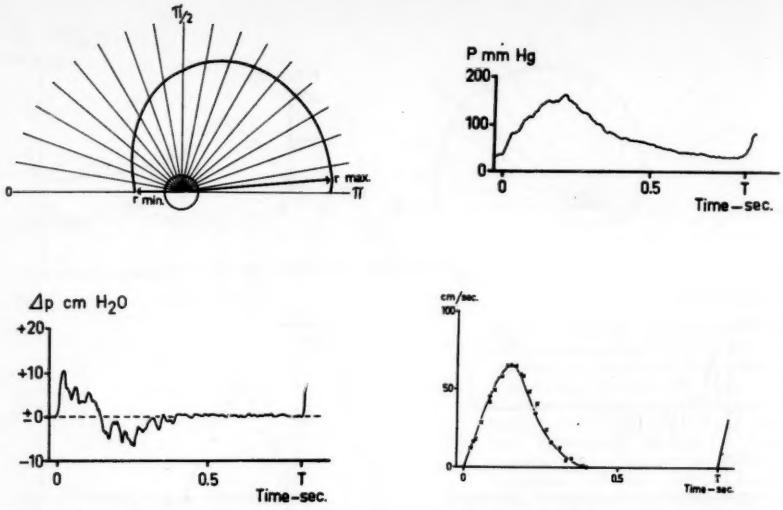


Fig. 4. Excentric with more smooth curvature and increased length of the ejection phase. Differential pressure and computed velocities arranged as before. The "stroke volume" is 66 cm³. Computed value 65 ± 4 cm³.

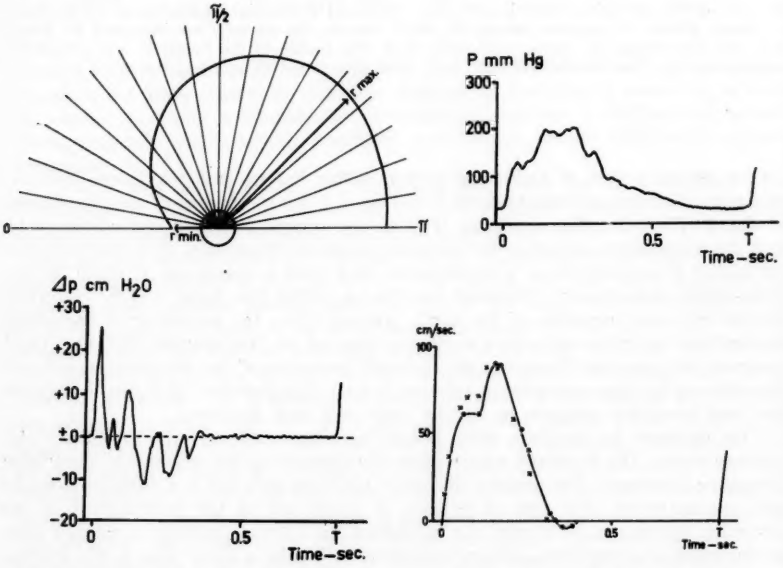


Fig. 5. Excentric with steep ascent and increased "stroke volume" (80 cm³). Computed value 81 ± 5 cm³.

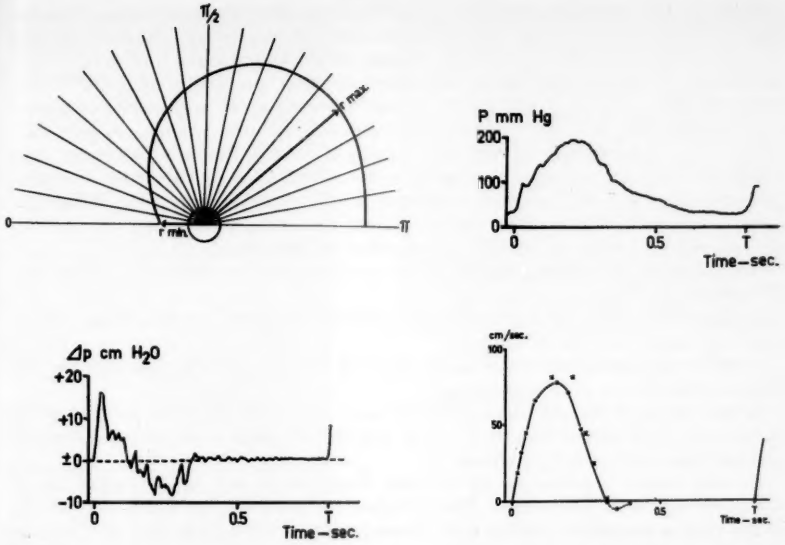


Fig. 6. The same excentric as in fig. 8 with less steep ascent. The duration of the ejection phase about the same. The "stroke volume" is 81 cm³. Computed value 76 ± 5 cm³.

Model experiments

In previous papers (PORJÉ and RUDEWALD 1955, 1957 a, b, 1959) we referred to experimental studies of the hydrodynamics in a circulation model.

The main features of the model circulation system have been described earlier. A brief description of the experimental conditions and some technical details will be given for the sake of completeness.

The output characteristics of the pump are determined by the profile of the eccentric. The "stroke volume", the "systolic" phase and the velocities in the ejection flow can thus be varied simply by using different profiles of the eccentrics. The r. p. m. of the eccentric is constant and the period T = 0.82 sec. The temperature of the circulating liquid is 18° C. An elastic rubber tube, length 25 cm, is attached to the outlet of the pump and connected to an elastic plastic tube with a plexiglass muff. The volume elasticity modulus of the rubber tube is determined experimentally to be 930 mm Hg. The double-lumina catheter is inserted in the elastic plastic tube through a branch in the distal part of the tube and positioned with the foremost opening just outside the outlet of the pump. The differential pressure transducer and the catheter is filled with boiled water and carefully aired before the insertion of the catheter. Static calibration is carried out for both sides of the manometer. The Δp-curve is recorded and then the catheter endings are shifted to opposite sides of the pressure transducer to test the symmetry. Only when the integrated absolute values of Δp and $-\Delta$p are nearly identical, is the difference pressure curve accepted as correct. Disturbances due to small vibrations may cause minor differences in the shape of the curves but as a rule it is possible to decide by inspection whether the Δp-curve is correct or not.

Series of Δp-curves are recorded with different profiles of the eccentric. The circulating liquid is water. In Fig. 3—6 the profile curve of the eccentric, the corresponding Δp-curves and computed velocities are demonstrated. From the graph of the eccentric the velocity of the plunger can be calculated for short intervals of time (0.022 sec) and values for the outflow velocity are plotted for comparison with values computed from formula (17). It is observed that the computed and actual values compare well.

The effect of the viscosity is studied by adding a viscosity increasing substance (methocel, ASTRA) to the water circulating in the model. The Δp-curves for kinematic viscosities ranging from 1—9 centistoke nave nearly the same shape. The differential pressure tends to be slightly positive as the viscosity increases, but values for this positivity are not precise enough to permit any quantitative estimation.

Our experimental observations from this particular model circulation system may be summarized thus:

1. Computed values for the instanteneous velocity and the "stroke volume" are in good agreement with given values.

2. When the outflow pattern from the pump is slightly varied the shape of the Δp-curve is changed in a distinct way.

3. The shape of the Δp-curve is in the main unaltered when the viscosity of the circulating liquid ranges between 1—9 cst and the deviation of the mean differential pressure from zero is not significant.

In these models experiments we find that fluid velocity and stroke volume can be computed with sufficient accuracy from formula (16) and (17). The pulse-wave velocity of the tube is determined from a static measurement of the volume elasticity modulus according to Hallock and Benson. From their measurement on autopsy material we find that this tube would correspond to a rather rigid aorta.

For kinematic viscosities ranging from 1—9 cst the mean Δp does not deviate significantly from zero. The viscosity is measured in an Ostwald viscometer at 18° C. At high rates of shear the blood and the fluid used here certainly behave differently.

According to the Poiseuille equation the pressure drop would be at most 0.1 cm H_2O in these experiments. An accurate determination of the mean Δp of this order of magnitude is not possible with the present apparatus. From the theoretical considerations referred to, the pressure drop due to the viscosity should be negligible for a short distance along the tube. Experimentally we arrive at the same conclusion.

Aortic differential pressure in human beings

In 1956 I. G. PORJÉ and B. RUDEWALD started to record the differential pressure in the human aorta and these records presented in 1957, were, as far as we know, the first direct records from human beings.

At that time we used two catheters which were inserted in the aorta after bilateral puncture of the femoral artery. Now the special double-lumen catheter is used for recording the aortic differential pressure. The catheter is inserted percutaneously in the femoral artery. The vessel is punctured under local anesthesia with a modified Gidlund cannula. The catheter is advanced to the ascending aorta under fluoroscopic control. The catheter is curved in its foremost part to facilitate its passage through the aortic arch (ÖDMAN and PHILIPSON 1958). Two perpendicular radiograms are exposed to check the position. From the radiograms the distance (L) between the catheter openings can be calculated. The catheter is perfused with heparinized saline

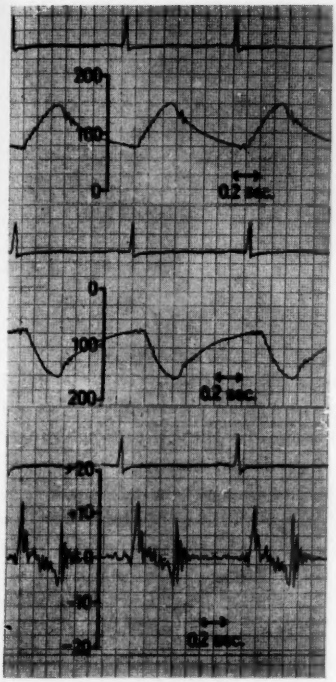


Fig. 7 a.

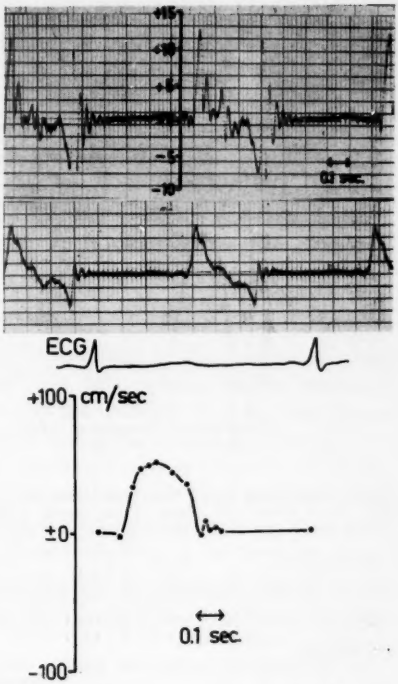


Fig. 7 b.

Fig. 7 a. Ascending aortic pressure (A and B) and differential pressure (c) in a 58-year-old woman with normal aortic valves. ECG above the curves.

Fig. 7 b. The differential pressure with greater amplitude and the time scale streched out. Lower curves is obtained by filtering off the higher frequencies. The maximum Δp value is about 12 cm H_2O. L = 8.5 cm. Values for the fluid velocity obtained from formula (17) are plotted in bottom diagram.

during the whole investigation. Records of the pressure and the differential pressure are only taken during short intervals of time. With these precautions there is no risk for coagulation at the catheter openings. Extreme care must be devoted to the airing of the system. As mentioned previously the symmetry of the system can be checked by shifting the end of the catheter to opposite sides of the differential pressure transducer. By suitable adjustment of the stop-cocks this test can be performed quite easily. With this technique the aortic differential pressure has been recorded in cases with normal aortic valves and with aortic valvular disease, particularly in aortic valvular stenosis. The total number of cases investigated with the differential pressure technique is 31. As the recording apparatus has been subjected to modifications during the

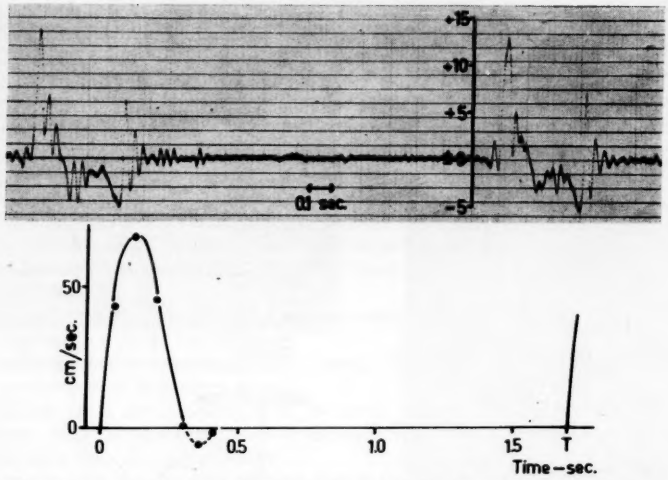


Fig. 8. Ascending aortic differential pressure (in cm H_2O) form a 64-year-old man with a total heart block. No signs of any aortic valvular disease, max. Δp is about 13 cm H_2O. L = 8 cm. Some values for the fluid velocity are plotted below (•).

course of the investigations the material cannot be treated statistically. The cases presented here are representative for the results obtained with our present technique.

Results

In cases with normal aortic valves the Δ p-curve from the ascending aorta has a characteristic pattern as shown in Fig. 7 a, b. At the onset of systole there is a distinct positive steep rise corresponding to the acceleration of the blood (the net force is directed *from* the heart). Then the Δp becomes negative and at the end of systole there is a negative dip followed by high frequency oscillations of very short duration. For negative Δp the blood is decelerated (the net force is directed *towards* the heart). During diastole the Δp is equilibrated to a smooth zero niveau. Fig. 8 shows another case with a total heart block. There were no clinical signs of any aortic valvular disease.

From formula (17) values for the blood velocity are obtained which we may first look upon as hypothetical. The velocity is assumed to be zero at the beginning of systole. There is a rapid increase of the velocity (Fig. 7 b, 8 b) to its maximum values of about 60—90 cm/sec. Then the velocity decreases to zero and this zero point agrees very well with the expected end of the ejection phase and the end of systole obtained from the ECG. During the remainder of the period (diastole) the velocity is nearly zero. In some cases small negative values are found just after the zero point. The stroke volume can be calculated if the cross-sectional area of the aorta is known *e. g.* from

thoracic aortography. Plausible values can be obtained from statistical tables (PORJÉ 1946). With such values we find that the stroke volume would be about 60—80 ml in the cases demonstrated here. The mean velocity is found to be about 12 cm/sec.

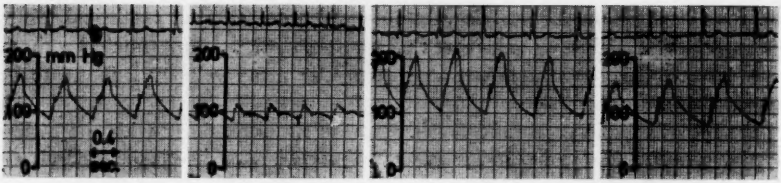
By recording the ascending aortic pressure simultaneously with the differential pressure, the heart power and the heart work can be calculated (I. G. PORJÉ and B. RUDEWALD 1957, formula 14). The distance L is measured from the radiograms to be about 8 cm in the cases demonstrated here. Further more we find that the mean value of the Δp is about zero — the deviation is not significant.

Discussion

With a distance of 8—10 cm between the openings in the catheter we get reliable differential pressure records and this distance also fits the length of the ascending aorta. As the foremost part of the catheter is curved this figure is reduced and it must be measured in each individual case in the radiograms. To compute hemodynamic data about the blood motion from our formula (7) and (13) the cross-sectional area of the aorta and its variation with time and the length coordinate must be known. Recent investigations by PETERSEN *et al.* on the dog's aorta with simultaneous recording of the cross-sectional area and the intra-vascular pressure indicate that the dynamic behaviour of the aorta is that of a rather rigid tube. It is reasonable to assume that the human aorta should behave in much the same way. This view is also supported by observations from angiographic studies of the ascending aorta made by ARVIDSSON (1960). He finds that the area change in cases with intact valves is at most 5 % for a normal range of pulse amplitudes. This indicates that the simplified formulae (16) and (17) could be used with sufficient accuracy to compute instantaneous mean flow and aortic blood velocity in such cases. The instantaneous blood flow at the root of the aorta should differ only slightly from the instantaneous mean flow. If sufficient experimental data are available formulae (7) and (13) can be used for more exact calculations of these data also in cases, where the cross-sectional area varies within wider limits.

The high frequency oscillation superimposed on the Δp-curve are mainly caused by rapid catheter vibrations. A slight change of the position of the catheter will alter the contents of these oscillations. When the Δp-curves from different positions are integrated for suitable intervals of time values are obtained that differ slightly. The high frequency response of the recording system should however be adapted to minimize these artefacts.

The maximim differential pressure is about 13 cm H_2O in the cases presented here. Then the maximum acceleration of the fluid would be about 1,600 cm/sec. The volume of the left ventricle has been calculated from rapid serial angiocardiography by ARVIDSSON (1960). In cases with intact mitral- and aortic valves the maximum acceleration of the blood during early systole



A	B	C	D
$P_s = 157$ mmHg	$P_s = 110$	$P_s = 204—195$	$P_s = 157$
$T = 0.65$ sec	$T = 0.51$	$T = 0.70$	$T = 0.80$
$P_d = 88$ mmHg	$P_d = 88$	$P_d = 100—95$	$P_d = 76$
$f = 92$ stroke/min	$f = 118$	$f = 85$	$f = 75$

Fig. 9. Aortic pressure just above the values during the Valsalva maneuvre. Record A taken immediately before the strain and record B during expiration (intraoral pressure about 50 mmHg) after a maximum inspiration. Record C is taken 5 sec. and recorded D 15 sec. after the release of the strain. The maximum systolic pressure is denoted by P_s and the end.-diastolic pressure by P_d. T is the period and f the heart frequency.

is calculated to be of the same order of magnitude as that obtained from the differential pressure record.

Of particular interest is the observation that the mean Δp does not differ significantly from zero. It seems justifiable to conclude that the viscosity forces are small compared to the inertial forces in the ascending aorta.

As mentioned above the differential pressure technique is particularly suitable for the study of rapid alterations of the dynamics of the arterial blood flow. To illustrate this we shall present some preliminary studies of the circulatory effect of the Valsalva manoeuvre and a rapidly acting drug. The use of the method for diagnostic purposes in aortic valvular stenosis has been dealt with earlier (PORJÉ and RUDEWALD 1959).

The differential pressure during the Valsalva Manoeuvre

The Δp-curves presented so far are obtained during a steady state. In order to study the Δp-pattern when the left ventricular output is changing rapidly the patients are instructed to carry out the Valsalva manoeuvre. The pressure just above the valves is recorded during the manoeuvre (Fig. 9). Some Δp-curves from a sequence of records are shown in Fig. 10 together with computed velocity curves. We find that the altering of the shape of the Δp-curve is characterized by a slightly increased amplitude at the beginning of the maoeuvre, followed by a strong decrease. When the patient starts to breathe regularly the Δp is for some strokes of slightly increased amplitude, but soon it returns to the basic amplitude. The relative change of the corresponding stroke volumes are given in Fig. 10.

When a small dose of a nitrite, 5—10 mg, (Etrynit, Bofors) is given to patients a transient decrease of the central blood pressure is observed in some cases. The aortic differential pressure and computed blood velocity for such a case

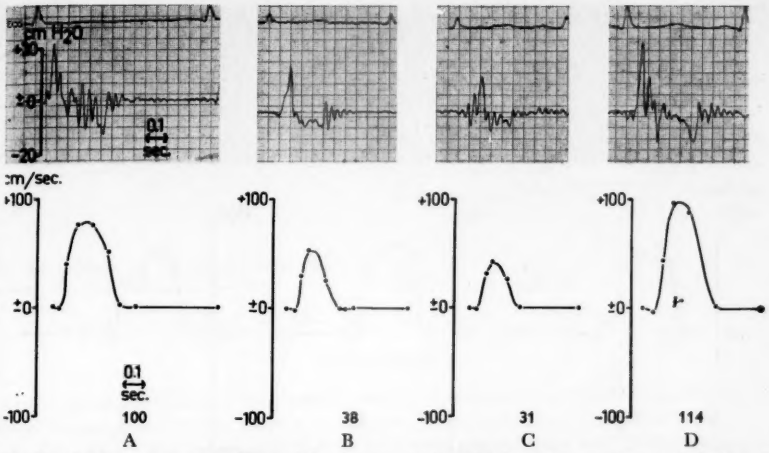
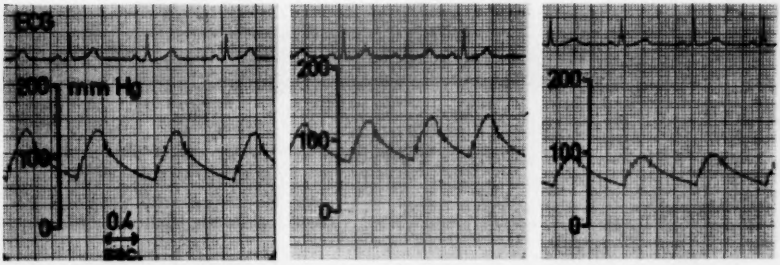


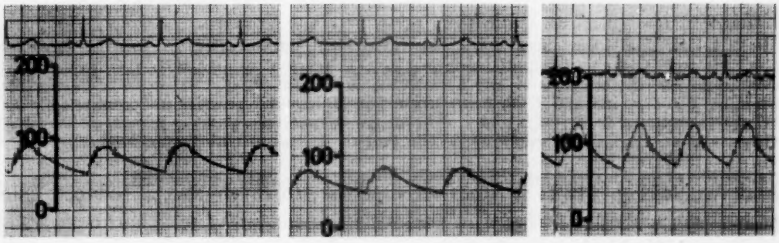
Fig. 10. Ascending aortic differential pressure during the Valsalva maneuvre. A before, B and C during strain and D 4 sec. after the release of the strain. Computed values for the blood velocity are shown below. The stroke volume before the strain is arbitrarily put to 100.



$P_s = 135$ mmHg $T = 0.89$ sec.
$P_d = 71$ mmHg
$f = 67$ stroke/min

$P_s = 124$ $T = 0.71$
$P_d = 70$ $f = 84$

$P_s = 90$ $T = 0.84$
$P_d = 56$ $f = 71$



$P_s = 86$ $T = 0.89$
$P_d = 48$ $f = 67$

$P_s = 84$ $T = 0.89$
$P_d = 50$ $f = 67$

$P_s = 133$ $T = 0.67$
$P_d = 70$ $f = 90$

Fig. 11 a.

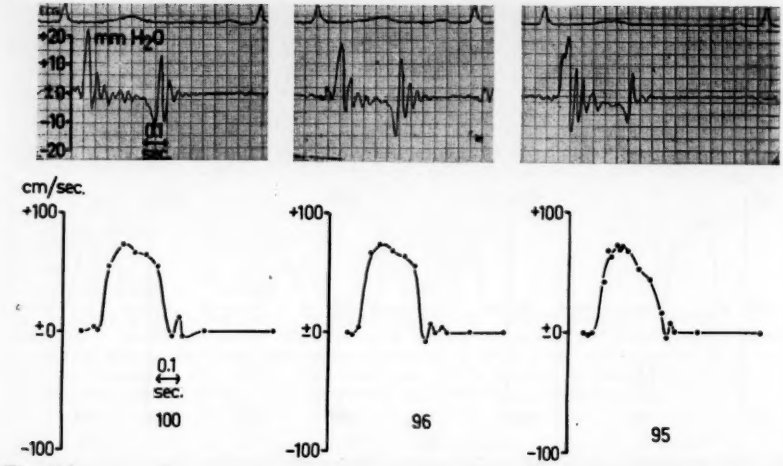


Fig. 11 b.

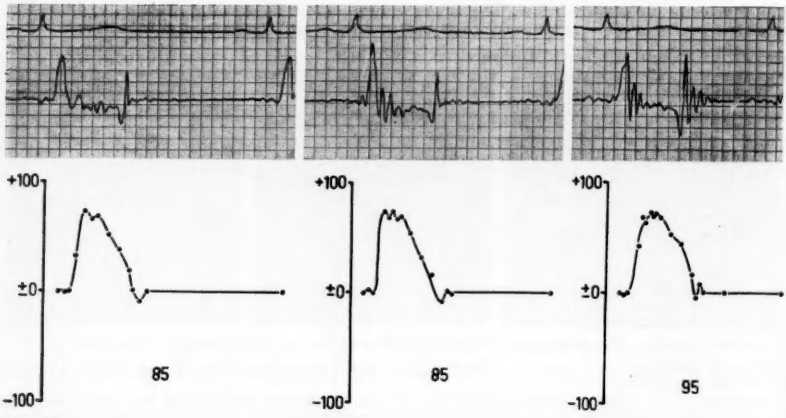


Fig. 11 c.

Fig. 11 a—c. The effect of a small dose of a nitrit, Etrynit, Bofors, upon the ascending aortic pressure and the differential pressure in a 48-year-old woman with pseudo-xanthoma elasticum. No clinical signs of any aortic valvular disease. P_s systolic pressure, P_d diastolic pressure, T the period and f the heart frequency. Differential pressure and computed blood velocity are shown in fig. 11 b and c. The stroke volumes are given in percent of the stroke volume before etrynit is administrated. The records are taken with 5 min intervals.

is shown in Fig. 11. It is observed that the stroke volume is reduced concomitantly with the decrease in the central blood pressure. Pharmacological studies of this kind are going on.

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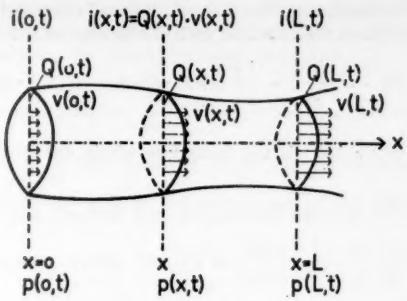

Fig. 12. Sketch of an elastic tube illustrating the symbols used. See text.

Addendum

In the theoretical treatment the ascending aorta is represented by a uniform, non-branching, elastic circular tube. For our purpose it is convenient to discuss two alternatives separately: A. The motion of a non-viscous fluid in an elastic, circular tube, and B. The motion of a viscous fluid in a rigid circular tube. The following symbols are used:

t	time
x	the length coordinate taken along the axis of the tube
L	the length of the tube
R	the radius of the tube
$Q(x,t)$	the cross sectional area of the tube
Q_o	the cross sectional area at a certain pressure *e. g.* the end-diastolic pressure
$\varepsilon(x,t)$	the relative change of Q defined by $Q(x,t) = Q_o\left[1 + \varepsilon(x,t)\right]$
$p(x,t)$	the lateral pressure
$v(x,t)$	the axial fluid velocity
$i(x,t)$	the rate of flow $i(x,t) = Q(x,t) \cdot v(x,t)$
ϱ	the density of the fluid
μ	the viscosity of the fluid
υ	the kinematic viscosity $\left(\upsilon = \frac{\mu}{\varrho}\right)$
ω	the angular frequency

The motion of a non-viscous fluid in an elastic, circular tube

I. We apply the momentum equation to the fluid motion in an elastic tube of length L. The relation between the rate of change of the momentum and the forces acting upon the fluids is given by the following vectorial equation (*e. g.* Müller 1932)

$$(1) \quad \frac{\delta}{\delta t}\int_S \varrho \cdot \vec{v} \cdot ds + \int_F \varrho \cdot v_n \cdot df \cdot \vec{v} = -\vec{P}$$

The integration should be carried out over the space S containing the fluid and its boundary surfaces F (Fig. 12).

The x-component of eq (1) is

$$(2) \quad \frac{d}{dt}\int_o^L \varrho \cdot Q \cdot v \cdot dx + \varrho\left[Q(L,t) \cdot v^2(L,t) - Q(o,t) \cdot v^2(o,t)\right] = Q(o,t) \cdot p(o,t) - Q(L,t) \cdot p(L,t)$$

For oscillatory flow L should be small compared to the wavelength. Then the axial component of forces from the tube walls can be neglected. With $i(x,t) = Q(x,t) \cdot v(x,t)$ eq (2) can be written

$$(3) \quad L\frac{di_m(t)}{dt} = \frac{1}{\varrho}\left[Q(o,t)\cdot p(o,t) - Q(L,t)\cdot p(L,t)\right] + \frac{i^2(o,t)}{Q(o,t)} - \frac{i^2(L,t)}{Q(L,t)}$$

where $i_m(t)$ is the mean flow within the tube $\quad i_m(t) = \frac{1}{L}\int_o^L Q(x,t)\cdot v(x,t)dx$

The quotient between the cross sectional areas at $x = o$ and $x = L$ is

$$\frac{Q(L,t)}{Q(o,t)} = \frac{1+\varepsilon(L,t)}{1+\varepsilon(o,t)} \approx 1 - \Delta\varepsilon \quad \text{where} \quad \Delta\varepsilon = \varepsilon(o,t) - \varepsilon(L,t)$$

As $\varepsilon(x,t)$ is positive and small compared to 1, then $\Delta\varepsilon << 1$ and terms multiplied by $\Delta\varepsilon$ can be neglected.

With $\Delta p = p(o,t) - p(L,t)$ and $\Delta i = i(o,t) - i(L,t)$ eq (3) becomes

$$(4) \quad \frac{di_m}{dt} = \frac{1}{\varrho L}\cdot Q(o,t)\,\Delta p + \frac{\Delta i[i(o,t) + i(L,t)]}{L\cdot Q(o,t)}$$

The equation of continuity for the tube is

$$(5) \quad \frac{d}{dt}\int_o^L Q(x,t)dx = \Delta i$$

The mean value of Q within $o \le x \le L$ is $\quad Q_m = \frac{1}{L}\int_o^L Q(x,t)dx$

Combining eq (4) and (5) we get after integration

$$(6) \quad i_m(t) = \frac{1}{\varrho L}\int_o^t Q(o,\tau)\cdot \Delta p(\tau)d\tau + \int_o^t\left[\frac{i(o,\tau) + i(L,\tau)}{Q(o,\tau)}\cdot\frac{dQ_m(t)}{d\tau}\right]d\tau + C$$

Where C is a constant to be determined from some boundary condition *e. g.* $i_m(o) = O$. Then $C = O$

Put $i_m(t) = \mathcal{J}_I(t) + \mathcal{J}_K(t)$ with $\mathcal{J}_I = \frac{1}{\varrho L}\int_o^t Q(O,\tau)\cdot \Delta p(\tau)d\tau + C$

and $\mathcal{J}_K = \int_o^t\left[\frac{i(o,\tau) + i(L,\tau)}{Q(o,\tau)}\cdot\frac{dQ_m(t)}{d\tau}\right]d\tau$ with $i(o,t) + i(L,t) = 2\mathcal{J}_I$ we get

$$(7) \quad i_m(t) = \mathcal{J}_I(t) + \int_o^t\left[\frac{2\,\mathcal{J}_I(\tau)}{Q(o,\tau)}\cdot\frac{dQ_m(\tau)}{d\tau}\right]d\tau$$

Comments

Eq (7) could be used for determination of the instantaneous mean flow through an elastic circular tube. What data from the ascending aorta are necessary for such a determination and how should they be obtained? The differential pressure, $\Delta p(t)$, must be recorded by some

suitable device for example by means of a catheter or a needle. The cross-sectional area $Q(o,t)$ and mean value $Q_m(t)$ must be determined by, for example, thoracic aortography. Then the non-corrected flow, $\mathcal{J}_I$, and the correctional term, $\mathcal{J}_K$, can be computed graphically.

II. Let us now suppose that the aortic pressure gradient, $\frac{\delta p}{\delta x}$, the cross sectional area of the aorta, Q, and its derivative with respect to x, $\frac{\delta Q}{\delta x}$ are measured at the root of the aorta viz. at $x = o$. Then the following equations can be used for the computation of the aortic blood flow.[1]

Combining the motion equation

(8) $$\frac{\delta v}{\delta t} + v \cdot \frac{\delta v}{\delta x} = -\frac{1}{\varrho} \cdot \frac{\delta p}{\delta x}$$ and the equation of continuity

(9) $$\frac{\delta Q}{\delta t} + \frac{\delta Q \cdot v}{\delta x} = 0$$ the following equation is obtained

(10) $$\frac{dU(t)}{dt} - \frac{U(t)}{(Q)_{x=0}}\left[\left(\frac{\delta Q}{\delta t}\right)_{x=0} + U(t)\left(\frac{\delta Q}{\delta x}\right)_{x=0}\right] = -\frac{1}{\varrho}\left(\frac{\delta p}{\delta x}\right)_{x=0}$$

where $U(t) = v(o,t)$

The functions to be measured are denoted by

$$f(t) = -\frac{1}{\varrho}\left(\frac{\delta p}{\delta x}\right)_{x=0} \; ; \; g(t) = (Q)_{x=0} \; ; \; h(t) = \left(\frac{\delta Q}{\delta x}\right)_{x=0}$$

The flow at $x = o$ is denoted by $F(t) = (Q)_{x=0} \cdot v(o,t) = g(t) \cdot U(t)$

The differential equation for $F(t)$ becomes

(11) $$F'(t) - 2F \cdot \frac{g'}{g} - \frac{F^2 \cdot h}{g^2} = f \cdot g$$

This equation is of the Riccalis type and it cannot be solved in an explicit form. For a rigid tube eq (11) is reduced to $F'(t) = f \cdot g$ with the solution $F = F_o(t)$

$$F_o(t) = \int_o^t f(\tau) \cdot g(\tau) d\tau - k_o$$

The constant of integration k_o can be determined from the condition that

$$\text{min. } F_o(t) = 0 \quad \text{then} \quad k_o = \text{min.} \int_o^t f(\tau) \cdot g(\tau) d\tau$$

The terms $2F \cdot \frac{g'}{g} + \frac{F^2 \cdot h}{g^2}$

can be looked upon as correctional terms. We get a good estimation of them by putting $F = F_o$.

[1] B. J. Andersson, personal communication.

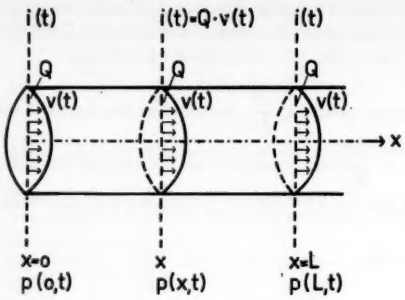

Fig. 13. See text.

Then eq (11) becomes

(12) $F' = f \cdot g + 2F_o \cdot \frac{g'}{g} + \frac{F_o^2 h}{g^2}$ Integration gives

(13) $F(t) = F_o(t) + \int_o^t \left[\frac{2F_o(\tau) \cdot g'(\tau)}{g(\tau)} + \frac{F_o^2(\tau) \cdot h(\tau)}{g^2(\tau)} \right] d\tau - k_1$ where

$k_1 = \min. \int_o^t \frac{2F_o(\tau) \cdot g'(\tau)}{g(\tau)} + \frac{F_o^2(\tau) \cdot h(\tau)}{g^2(\tau)} d\tau$ The stroke volume is

(14) $V_s = \int_o^T F(t)dt$ where T is the period.

To determine the efficiency of the pump (the heart) we have to measure the pressure $p(o,t)$ at $x = o$ (the root of the aorta).

The mean effect during one period is

(15) $\mathrm{E} = \frac{1}{T} \int_o^T p(O,t) \cdot F(t)dt$

Comments:

If the second correctional term $\frac{F^2 h}{g^2}$ is neglected eq (13) becomes formally identical with eq (7). The functions $g(t)$ and $h(t)$ can be determined in the manner indicated above and if the pressure gradient $\frac{\delta p}{\delta x}$ is substituted by the differential pressure over a short distance L, $\frac{\Delta p}{L}$, then eq (13) offers possibilities for a more exact calculation of the flow.

Discussion:

For a *rigid tube* eq 7 is reduced to $i(t) = Q \cdot v(t) = \int_o^t \Delta p(\tau)d\tau + C$

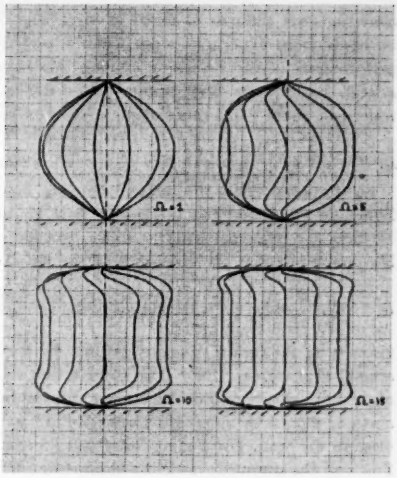

Fig. 14. Velocity profiles for the oscillatory flow of a viscous, non-compressible fluid for a range of values of Ω. See text. (From WELANDER 1950).

viz. the integration of the pressure difference will give values proportional to the instantaneous fluid velocity, $v(t)$, and as Q is a constant (Fig. 2) also to the instantaneous flow, $i(t)$, through the tube. The distance L can be chosen arbitrarily. The shortest L, that can be obtained is determined by the limitations of the recording system.

For a *rather rigid tube* and within a restricted range of variations of the pressure amplitude the difference between in- and outflow, Δi, is a small quantity for a short part of the tube. Then the correctional terms can be neglected and the mean instantaneous flow through the tube, $i_m(t)$, can be computed from

$$(16) \quad i_m(t) = \frac{1}{\varrho L} \int_o^t Q(O,\tau) \cdot \Delta p(\tau) d\tau + C$$

and $i_m(t)$ differs slightly from the inflow $i(o,t)$. The linear velocity $U(t)$, obtained from

$$(17) \quad U(t) = \frac{1}{\varrho L} \int_o^t \Delta p(\tau) d\tau + C$$

will approximate to the mean instantaneous fluid velocity. It differs more or less from the fluid velocity in the entrance flow, $v(o,t)$, but the deviation should be within rather close limits. The distance, L, should be as short as possible.

If the tube is more elastic and/or the pressure amplitude varies within wider limits, the difference between in- and outflow, Δi, cannot be neglected even for a short distance L. In this case the general equations must be used for computation of the mean flow $i_m(t)$. The flow at a particular point *e. g.* $i(o,t) = F(t)$ can at least in principle be computed according to eq (13) if L is sufficiently small.

The anatomy of the ascending aorta does not allow the pressure difference to be measured over a distance greater than about 8—10 cm. The measuring points should be referred to the centre of the cross-sectional to reduce the effect of the curvature of the vessel. In practice these

conditions are difficult to ful-fil in vivo. The choice of a particular L should, therefore, first be made from experiments particularly arranged to test the validity of the theory and the performance of the recording system. We have preferred to do this in a model circulation system. Experiences from studies of the dynamics of the aortic blood flow and the vascular walls will show what measuring parameters should be used.

B. The motion of a viscous fluid in a rigid circular tube

For stationary flow the relation between flow and pressure drop is given by the wellknown Poiseuille equation. For oscillatory flow equations have been deduced by several research workers. We refer, particularly, to the excellent and extensive theoretical studies by Womersly. He points out that the character of the flow is determined by a non-dimensional parameter $\alpha = R\sqrt{\frac{w}{\nu}}$ where R is the radius of the tube, w is the angular frequency and ν is the kinematic viscosity of the liquid. Velocity profiles for a range of values of α have been given by, for example, WELANDER (1950) (Fig. 14). He uses the symbol Ω instead of α. For increasing values of Ω the velocity profile becomes more flattened in the axial part of the tube. In the human aorta Ω should be about 20. Then the fluid is mainly unsheared, except for a rather thin layer close to the wall. For a given tube and a given kinematic viscocity the phase lag between the flow and the pressure gradient will approach 90° as the frequency increases (WOMERSLEY 1955).

FRY *et al.* (1956) has derived a formula from the Navier-Stoke differential equation for the computation of the flow in the ascending aorta from the pressure difference. These assume that the frictional forces are proportional to the velocity of the fluid. Recently FRY (1959) proposes a formula with certain tentative constants. Proportionality between frictional forces and fluid velocity is also treated by WELANDER (1950) who gives an equation for the computation of the constant for different types of flow.

Comments

The application of these results to the flow near the outlet of the pump (the ascending aorta) is questionable. Even in pulsatile flow the velocity profile does not form until a certain unknown distance from the tube inlet. The velocity profile may be determined mainly by the velocities in the ejection flow and turbulence may occur. For an elastic tube the situation becomes even more complex and there is no general solution yet to the non-linear differential equations valid for this type of flow. Therefore, it seems to be justified to use the formulae for a non-viscous flow to compute the blood flow in the ascending aorta in man. The errors due to viscous effects should be of minor importance compared to those from other sources. There is also experimental evidence that supports this view (see page 8). The pressure loss due to the visco-elastic properties of the aortic walls are another possible source of error. For an estimation of this effect the studies on the dog's aorta by PETERSEN *et al.* (1960) are of particular interest (see page 11).

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Effects of Motor Inactivation on the Chemical Sensitivity of Skeletal Muscle

By

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Abstract

JOHNS, T. R. and S. THESLEFF. *Effects of motor inactivation on the chemical sensitivity of skeletal muscle.* Acta physiol. scand. 1961. *51.* 136—141. — In order to determine whether the lack of motor nerve impulses, without denervation *per se*, alters the chemically sensitive area in skeletal muscle, the size of the ACh-sensitive areas in muscle fibres of cat tenuissimus were measured after isolation of the motor neurones by severance of the dorsal roots below a lower lumbar cord transection. The sensitivity of the individual muscle fibre to ACh was determined by iontophoretic micro-application of the drug, and by intracellular recording of the potential change. The frequency of miniature end-plate potentials, their amplitude, and their time course, were relatively unaffected by the isolation procedure. The procedure also caused little increase in the size of the ACh-sensitive surface. Therefore, inactivation *per se* did not alter the chemically sensitive area.

Following denervation the chemically sensitive area of the motor end-plate increases in size, extending progressively from the end-plate toward the tendons at either end of the fiber. Within one week the entire muscle membrane becomes chemically sensitive (AXELSSON and THESLEFF 1959). Experiments with botulinum toxin have indicated that lack of transmitter release, and not nerve degeneration itself, is primarily responsible for initiating the process which produces the high and uniform chemical sensitivity of chronically denervated muscle

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(THESLEFF 1960 a). However, the possibility that inactivity *per se* is responsible for the spread of chemical sensitivity deserves consideration, particularly since CANNON and HAIMOVICI (1939) have reported that an increased sensitivity to acetylcholine (ACh) follows decentralization of motor neurones in the cat.

The present study to investigate whether the lack of motor nerve impulses alters the chemically sensitive area in skeletal muscle, consisted of determining the size of the ACh-sensitive areas in muscle fibres of the tenuissimus muscles of the cat. Such determinations were made before, and at varying periods after, the elimination of all afferent impulses playing upon the lower motor neurones of the subserved tenuissimus muscle. The isolation of the motor neurones was accomplished by severance of the dorsal roots below a cord transection, as described by TOWER (1937 a, b).

Methods

Complete isolation of the lower motor neurones serving the tenuissimus muscles was successfully accomplished in 9 cats. It had previously been established that the tenuissimus muscle of the cat receives its motor nerve supply through the seventh lumbar and the first spinal roots. Under pentobarbitone anesthesia, the spinal cords were transected between segments L 4 and L 5, and all dorsal roots below the transection were identified and severed. Furthermore, all ventral roots below S 3 or 4 were severed. In such an isolated preparation, the ventral horn cells remain intact, yet deprived of all afferent impulses (TOWER 1937 a, b, ECCLES 1944).

Despite the severe operative procedure and its deficit, it was possible to maintain the animals in relatively good condition for periods of from one to four weeks, the weight loss being 20—30 % or less. In three cats a "sham" operation was performed, *i. e.*, only the actual isolation of the cord segments was omitted. At intervals of two days the muscles of the hindlegs were tested for the presence of electrical activity by the use of conventional electromyography with concentric needle electrodes.

In individual muscle fibers the sensitivity to ACh was determined by iontophoretic micro-application of the drug, and by intracellular recording of the resultant membrane potential change. (For details of the experimental apparatus, and the techniques for drug application and recording, see AXELSSON and THESLEFF 1959, and THESLEFF 1960 a.) As ACh was released by a current pulse from the micropipette, that part of the muscle membrane at which the drug produced a depolarization, with a latency of less than 10 msec., was considered sensitive to the drug. The length of the sensitive region was measured with a binocular microscope with an eye piece scale at 80 × magnification. To avoid the use of muscle fibres in which the motor nerve might have degenerated as a consequence of the operative procedure, the end-plate region was always located and examined for the presence of minature end-plate potentials (m.e.p.p.'s). The m.e.p.p.'s were photographed on single sweeps, and their mean frequency calculated from at least fifty m.e.p.p.'s from each fiber.

Since a close correlation has been shown to exist between the size of the ACh-sensitive area in a muscle, and the magnitude of contracture which is produced by diffusely applied ACh (ELMQVIST and THESLEFF 1960), each of the muscles examined by the microelectrode technique was subsequently studied to determine its isotonic shortening in response to graded doses of ACh. One to four weeks after the operation both tenuissimus muscles were removed from each cat, then tested for their sensitivity to applied ACh.

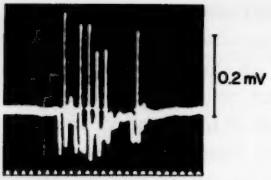

Fig. 1. Motor unit potentials recorded from the hindleg of a cat 18 days after lower motor neurone isolation procedure. The time marking is 4 msec.

Results

Immediately following the surgical isolation of the cord segments, both hindlegs were completely inactivated, as shown by electromyography and the obvious observation of paralysis. After one week of inactivity, however, spontaneous intermittent fasciculations were observed, and they were identified as motor unit potentials (Fig. 1). This spontaneous motor neurone activity persisted throughout the remainder of the observation period, *i. e.*, up to three weeks.

The frequency of m. e. p. p.'s was relatively unaffected by the isolation of the segmental lower motor neurones (Table I). Neither was their amplitude nor time course altered.

When the length of the ACh-sensitive surface in a single muscle fibre is determined by iontophoretic micro-application of the drug, records such as those in Fig. 2 are obtained. The highest sensitivity is observed at the visible end-plate region (records D and E), and from that point the local sensitivity falls rather symmetrically toward both ends. For example, in Fig. 2, the total length of the muscle fibre which responds to ACh is 0.5 mm. The mean lengths of ACh-sensitivity in control muscles and in muscles with isolated motor neurones are shown in the last column of Table I. It is evident that the length of the fibre sensitive to ACh is not markedly increased by one week of complete inactivation. After a longer period, a moderate increase of the ACh-sensitive

Table I. Mean values and standard deviations for the frequency of m.e.p.p.'s and the length of the ACh-sensitive surface in single fibres of cat tenuissimus muscle. The values are recorded according to the duration of the period of muscle inactivity producted by surgical isolation of the motor neurones

Duration of inactivity (days)	Number of muscles	Number of end-plates	Mean frequency of m.e.p.p.'s (per sec)	Mean length of receptor surface (mm)
Control	6	35	14.3 ± 1.18	0.67 ± 0.019
7	6	30	7.4 ± 0.80	0.81 ± 0.027
12—13	6	29	5.3 ± 0.68	(1.16)[1] range 0.5—> 2.5
18—27	6	24	22.1 ± 3.80	(1.04)[1] range 0.5—> 2.5

[1] For technical reasons distances longer than 2.5 mm could not be measured.

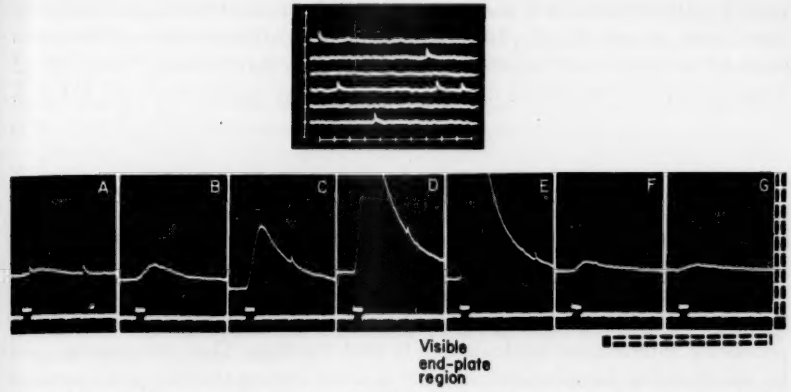

Fig. 2. Records illustrating how the length of the ACh-sensitive surface in a single muscle fibre is determined by iontophoretic release of the drug from a micropipette (currents through the pipette are registered in the lower tracing), and by intracellular recording of the resultant membrane depolarization (upper tracing). The records were obtained when ACh was released close to the fibre membrane at points increasing by 0.1 mm increments from the visible end-plate region. The m. e. p. p. frequency was recorded on successive single sweeps as shown in the upper record. The time marking is 20 msec; voltage calibration, 1 mV; monitor calibration (voltage scale), 2×10^{-8} A.

area occurred and, as shown by the range of measurements, the chemically sensitive length of a fibre occasionally exceeded 2.5 mm (for technical reasons distances longer than 2.5 mm could not be measured).

A muscle in which the chemically sensitive surface is enlarged will respond to diffusely applied ACh with a contracture (Axelsson and Thesleff 1959, Elmqvist and Thesleff 1960). ACh produced an isotonic shortening which, when the period of motor neurone isolation had lasted longer than one week,

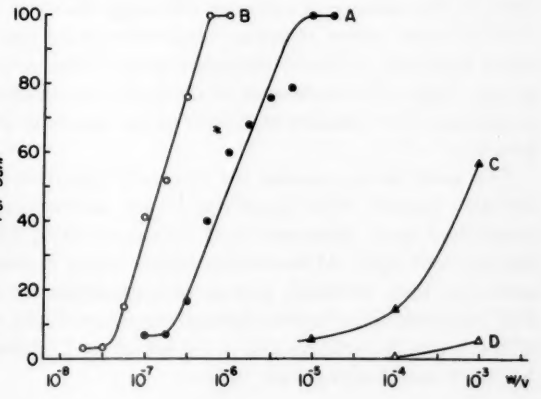

Fig. 3. Dose-response curves for ACh obtained in the cat tenuissimus. The isotonic shortening produced in the muscle by diffusely applied ACh is expressed in per cent of the shortening caused by rapid immersion of the preparation in isotonic K_2SO_4 solution. Curve A was obtained 14 days after motor denervation; B, 34 days after botulinum intoxication; C, 13 days after isolation of lower motor neurones; D, after "sham" operation (control).

was 20—50 per cent of that observed in a chronically denervated, or botulinum intoxicated, muscle (Fig. 3). However, the relative ACh-sensitivity of the membrane of the inactivated muscle was much less than that following denervation. That is shown by the shift of the dose-response curve toward the right in Fig. 3.

Discussion

Surgical technique was used which permitted isolation of the lower motor neurones without damaging them (TOWER 1937 a, b, ECCLES 1944). During the first post-operative week no sign of motor neurone activity was recorded. Subsequently, however, the motor neurones spontaneously became active, producing intermittent fasciculations in both hindlegs. Thus, the muscles may be considered to have been completely inactive during the first post-operative week, but later some spontaneous motor activity was present.

Following motor nerve degeneration in the cat the spread of the receptor surface from the end-plate region toward the tendons reaches its maximum extent by covering the entire fibre within seven days (AXELSSON and THESLEFF 1959). The present investigation has shown that complete inactivation of a muscle with an intact motor unit for the same period of time causes little increase in the size of the ACh-sensitive surface of the end-plate. Therefore, inactivity alone cannot be responsible for extension of the receptor surface after nerve degeneration. MILEDI (1960) has reached the same conclusion while working with incompletely denervated frog muscle. In sartorius fibres, with at least two end-plates, he observed that section of the nerve to one of them caused a spread of chemical sensitivity in the region of the latter end-plate, despite the fact that impulse transmission continued through the intact junction.

However, if lack of transmitter release were primarily responsible for the increase in ACh-sensitivity in chronically denervated muscle (THESLEFF 1960 b), the absence of a change following inactivity is understandable. Isolation of lower motor neurones abolishes synchronous transmitter release by nerve impulses, yet leaves the spontaneous transmitter activity (*i. e.*, the m. e. p. p.'s) intact. The continuous subthreshold transmitter release may be sufficient to maintain the chemical sensitivity of the muscle in about its normal area and intensity.

The main factor causing the increased sensitivity of denervated muscle to diffusely applied ACh appears to be an increase in the chemically sensitive membrane area (AXELSSON and THESLEFF 1959, MILEDI 1960). The slight enlargement of the ACh-sensitive surface which is observed after prolonged inactivation may, therefore, provide an explanation for the rather moderate (tenfold) supersensitivity to intra-arterial injections of ACh observed after transection of the lower thoracic portion of the spinal cord (CANNON and HAIMOVICI 1939, SOLANDT and MAGLADERY 1942).

We wish to thank Dr. BIRGITTA HOLMQVIST of the Institute of Physiology for showing us the methods for the isolation procedure. This investigation was aided in part by United States Public Health Service research grant B-2646 from the National Institute of Neurological Diseases and Blindness, and by grants from the Muscular Dystrophy Associations of America, Inc., and the Air Research and Development Command, United States Air Force, through its European Office. Unfailing technical assistance continued to be provided by Miss E. ADLER.

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Influence of Noradrenaline on Spinal Interneuron Activity

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Abstract

Skoglund, C. R. *Influence of noradrenaline on spinal interneuron activity.* Acta physiol. scand. 1961. *51.* 142—149. — The effects of intra-arterial and intravenous injections of noradrenaline on spinal interneurons have been studied on decapitate, curarized cats by means of intracellular technique. The most common effect, observed at a threshold dose of 1 μg per kg bodyweight, was an increase in excitability. Interneuron responses to single afferent volleys were facilitated, a typical effect being transformation of single spike responses into repetitive discharges. In the absence of any induced afferent stimulation noradrenaline injections were observed to set up discharges in initially silent cells and to cause an increase in frequency of prevailing activity, sometimes followed by a decrease. An initial decrease in excitability was only occasionally seen.

Introduction

The influence of adrenaline on spinal reflexes was shown by Schweitzer and Wright in 1937, and their finding of a modifying action of adrenaline on central transmission processes has since been confirmed in several investigations (cf. Paton 1958). After the demonstration of noradrenaline as a normal constituent in the animal body it was only natural that interest should be directed toward the possible action of this substance on the central nervous system and, as appears from Euler's review (1956), this problem has been approached along several lines. In general, similar effects have been found as for adrenaline. Spinal reflexes in the decapitate cat were thus found to be influenced in the same way by noradrenaline as by adrenaline, the most common effect being a facilitation of monosynaptic extensor reflexes, which appeared at about the same threshold doses for both substances when injected intra-arterially (Bernhard and Skoglund 1953, cf. Skoglund 1952). In a study of the functional

properties of spinal interneurons (KOLMODIN and SKOGLUND 1954) noradrenaline was observed to influence the discharge rhythm of interneurons in the ventral horn. CURTIS *et al.* (1957), using narcotized and fully deafferentated cat preparations, only occasionally observed potentiating effects of noradrenaline (or adrenaline) on monosynaptic reflexes and also reported that none of these substances had any effect on spinal interneurons of the Renshaw type.

A more detailed analysis of the noradrenaline effects observed in non-anesthetized cats has since been made and a brief description of the modifying action on interneuron activity seemed justified, particularly in view of some recent observations of a modulating action of noradrenaline on unit activity in the reticular formation (BRADLEY and MOLLICA 1958) and at the receptor level (LOEWENSTEIN 1956).

Methods

Decapitate, slightly curarized cats (average weight 2.8 kg) kept on artificial ventilation have been used. Laminectomy was performed in the lumbosacral region and the ventral roots L_5—S_1 were usually cut to permit antidromic stimulation. In some of the preparations dorsal roots or peripheral nerves were prepared for electrical stimulation, while other preparations were kept as intact as possible for the purpose of studying interneuron activity under as normal conditions as possible. The intravenous injections were made into the jugular vein; for intra-arterial injections the method of HOLMSTEDT and SKOGLUND (1953), using a polyethylene catheter inserted in the aorta, was applied.

In most experiments Macrodex infusions were made to maintain the blood pressure; occasional checkings of the systemic arterial pressure gave values between 90 and 120 mm Hg. In a preliminary series of experiments the blood flow in the spinal cord was studied by means of a thermocouple inserted in the lumbar cord.

Capillary microelectrodes filled with 2.7—3.0 M KCl solution were used in connection with an input stage of cathode follower type. The main direct coupled amplifier, connected to the oscilloscope, also fed an inkwriter for continuous recording of the membrane potential. Part of the analysis of spike frequency variations was made on tape recordings from the actual experiments (for a detailed description of the recording technique see HAAPANEN *et al.* 1958).

The technical difficulties encountered in microrecording from central nervous structures when injecting substances with effects on circulation are obvious. The fact that even minute movements of the cord tissue in relation to the electrode tip are reflected in alterations in the recorded membrane potential level leads to several kinds of difficulties. Thus, it was found that injection of a few ml of Ringer's solution into the aorta may result in abrupt changes in the recorded membrane potential, apparently due to tissue dislocation caused by changes in the regional arterial pressure. With intravenous Ringer injections such changes were only occasionally observed. This type of artifact is recognized by its appearance immediately after the injection. The specific blood pressure changes due to noradrenaline develop more slowly and may result in later occurring dislocation effects. In certain cases abrupt or more slowly developing changes occurred in the membrane potential level at latencies varying from 10 to 30 sec after the intravenous injections and it is likely that these were secondary to blood pressure changes, the maximum rise of which occurs within these time periods. In such cases the membrane potential could often be restored to its initial level by a slight tap on the electrode holder. Variations in the recorded membrane potential due to dislocation do not influence the discharge frequency if the injury of the cell is not too large (cf. HAAPANEN *et al.* 1958).

Results

For the testing of noradrenaline effects interneurons were selected that had shown a steady membrane potential and constant activity level or unchanged responses to stimulation during a sufficiently long period (usually 5 min) to allow a safe estimation of the relatively slowly developing effects. The twenty-five interneurons thus selected represented cell types varying both with respect to functional connections and anatomical location.

In spite of the heterogeneousness of the cell population tested, the effects of an increased noradrenaline content of the blood — whether caused by intra-arterial or intravenous injections — were fairly uniform, the most commonly observed initial result being an increase of excitability. The threshold dose of intravenous injection was 3 μg, corresponding to about 1 μg per kg bodyweight. More marked effects were obtained by doses of 2—3 μg/kg. Injections of still higher doses were generally avoided because of the dislocation effects (cf. above). With intra-arterial injections of corresponding doses the effects were generally more pronounced.

In one series of experiments the noradrenaline effects were tested on the interneuron response to single shock stimulation of afferent nerves and various types of facilitation effects could be observed. Thus in one preparation, in which interneurons in the ventral horn were studied, a cell was observed which initially responded to an afferent volley in the gastrocnemius nerve with a typical single spike at threshold and maximally two spikes at suprathreshold stimulation. Some minute after intravenous injection of 5 μg noradrenaline this response changed into a train of 4—5 spikes, which effect slowly subsided during the following five minutes of observation of the cell. Another cell tested responded initially to the afferent muscle nerve volley only under simultaneous facilitatory influence from a skin area. After noradrenaline the cell responded readily to muscle afferent stimulation alone. Another manifestation of an increased excitability was a greater constancy of the response at threshold stimulation observed in certain cells.

The majority of the observations were made on cells in the absence of any afferent stimulation. Under such conditions some cells are initially silent, while others show a continuous discharge. In cells of the first type, noradrenaline injection was often observed to initiate discharges which varied from trains of impulses of some seconds' length up to several minutes' continuous firing. The impulse frequency kept within the range of the naturally occurring discharges, and high-frequency bursts of injury type were never recorded. Most of the cells of the second type, showing spontaneous discharges from the beginning, were also found to be influenced by noradrenaline. In some cases only slight effects could be observed, in the form of a change to a more irregular rhythm without any more pronounced variations in the average frequency, but in others definite changes in discharge frequency could be demonstrated. The initial

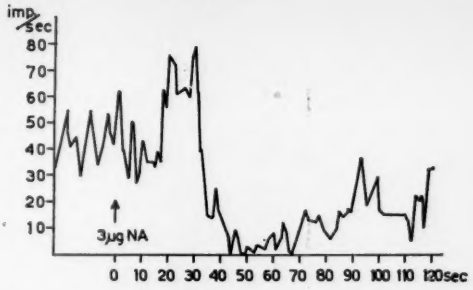

Fig. 1. Effect of noradrenaline on discharge frequency of spinal interneuron. Time in sec from moment of intra-arterial injection (at arrow).

change was usually an increase in frequency, and a slowing-down of the rhythm was only occasionally observed as an immediate effect. A depressive effect may also follow the initial facilitatory phase. A typical example of such a frequency modulation of interneuron activity is given in Fig. 1. The cell had first been under observation for a period of 10 minutes, during which it had been discharging at an average frequency of 45 per sec, with maximal fluctuations between 30 and 55 per sec. An intra-arterial injection of 3 µg noradrenaline resulted in a definite increase in frequency up to 75—80 per sec. As appears from the figure, this period of increased activity, lasting some 15 sec, was followed by a period of decreased frequency from which the cell slowly recovered during the second minute after the injection.

Some of the units tested were unaffected by noradrenaline. However, on the basis of the present rather limited material it is not possible to judge whether the variations in noradrenaline effects are due to different reactions of various cell types. Adrenaline was tried only in a few instances but also seemed to have a facilitatory action.

In several cases a small but definite decrease of the membrane potential was recorded simultaneously with the increase in discharge frequency, and this may represent a true membrane potential change (cf. KOLMODIN and SKOGLUND 1958). However, in most cases there was no direct relationship between potential level and frequency through the whole sequence of variations induced by an injection of noradrenaline. An intermingling of dislocation effects (cf. Methods) and true membrane potential changes is the most obvious explanation of such discrepancies, and before artifact changes can be safely excluded nothing can be deduced about a possible specific membrane effect of noradrenaline.

Discussion

The results described above imply that injections in non-anesthetized cat preparations of relatively small doses of noradrenaline — 1 µg per kg bodyweight — are reflected in changes of single interneuron activity. For comparison, the normal noradrenaline content of the blood is about 1 µg per litre blood

plasma and the average resting secretion in chloralose-anesthetized cats has been found to be about 0.4 μg per kg bodyweight per minute, calculated on both adrenal glands (EULER and FOLKOW 1953). As an example of activity secretion may be taken the average value obtained by EULER and FOLKOW during splanchnic stimulation, 1 μg/kg/min per adrenal gland. The blood content variations brought about by the present experiments may thus be said to approach the physiological range, although such sudden changes as those induced by injections do not occur normally.

For reasons given below, the interpretation of the results as being due to a direct action of noradrenaline on the nerve cells is the most likely; however, in view of the experimental conditions this conclusion can be reached only by excluding certain indirect modes of action, well-known from similar argumentations by previous investigators in this field. Thus, since a comparatively intact preparation was used in the present experiments, so as to be able to study interneuron activity under conditions as normal as possible (cf. KOLMODIN 1957), changes in afferent inflow may be an interfering factor. HUNT's (1960) finding that discharges from muscle spindles are influenced by sympathetic hormones indicates one possible source of error of this kind, but this mechanism seems less probable, if one may judge from the large doses of intravenously injected adrenaline, 40—80 μg/kg, which were necessary to produce significant changes in the afferent inflow. WILSON's (1956) attempts to demonstrate any changes in the afferent inflow in the dorsal roots of cats after adrenaline injections were also negative.

That circulatory changes due to the blood pressure variations induced by adrenergic substances constitute a significant factor in their action on central nervous activity has been rejected by most authors (*e. g.* SCHWEITZER and WRIGHT 1937, WILSON 1956, BRADLEY and MOLLICA 1958). KISSEL and DOMINO (1957) reported that the effects of adrenergic substances on the patellar reflex were maintained after stabilization of the blood pressure by means of a reservoir system and also showed that a mechanical elevation of the pressure failed to reproduce the effects. However, it should be pointed out that if the systemic arterial pressure is abnormally low from the beginning, as may be the case in decapitate preparations when necessary precautions to maintain the blood pressure have not been taken, the spinal neurons may be apt to respond to an improved circulation (cf. KOLMODIN 1957). VOORHOEVE (1960) considered the adrenaline and noradrenaline effects observed on fusiform motoneurons in decapitate cats to be due to an increase in spinal cord perfusion, since effects were only occasionally observed in decerebrate cats with arterial pressure above the critical level.

Regional changes in spinal cord circulation have also to be taken into account. As appears from SOKOLOFF's (1959) review several studies of noradrenaline effects on brain circulation have been performed, some of which indicate that the cerebral vessels are constricted to a higher degree than the over-

all circulatory bed, but corresponding investigations on spinal cord seem to be lacking. FIELD *et al.* (1951), using thermocouple technique for detection of blood flow changes in the rabbit's spinal cord, observed however that active vasoconstriction in the lumbar cord may result from adrenaline injections. In a preliminary series of experiments on decapitate cats, using the same technique, similar effects have been observed after noradrenaline injections; of special interest was the finding that after intra-arterial injection regional blood flow changes in the spinal cord could be observed in the absence of any more pronounced systemic effects (SKOGLUND, unpublished observations). To be able to judge of a possible vascular influence it seems that the excitatory effects should rather be correlated to regional blood flow changes than to variations in systemic arterial pressure. The relatively long latencies of the facilitatory effects of noradrenaline might point to a secondary action via regional vascular changes, but a slow reaction of the nerve cells to noradrenaline is also a possible explanation, particularly if metabolic changes are involved (cf. below).

In strong support of a direct action of noradrenaline on the nerve cells speak the findings of similar effects at the receptor level. As demonstrated by LOEWENSTEIN (1956), the facilitatory action of sympathetic stimulation on the frog's touch receptors can be reproduced by application of noradrenaline or adrenaline, and the similarities between the effects on these receptors and on the spinal interneurons are striking. Thus a touch receptor which ordinarily fired only one impulse in response to a mechanical stimulus discharged repetitively after application of sympathetic hormones, and in the absence of stimulation spontaneous firing could be initiated or increased.

However, from such analogies no conclusion should be drawn as to a transmitter function of noradrenaline in the central nervous system such as postulated at sympathetic nerve endings. It might rather be suggested that noradrenaline, as well as adrenaline, constitutes one factor relevant to the excitability level in nerve cells and other excitable structures. With respect to adrenaline some recent results seem to indicate that such an action may occur both via an influence on membrane permeability and via metabolic changes (cf. AXELSON *et al.* 1959). The latter alternative implies that the direct action of sympathetic hormones and their secondary effects due to vascular changes may be intimately related.

The reactions of spinal interneurons to an increased noradrenaline content in the blood are strikingly similar to those observed in units of the reticular formation (BONVALLET *et al.* 1956, BRADLEY and MOLLICA 1958), and it is possible that many types of central neurons show such a sensitivity, although to a varying degree. A further analysis of the action of sympathetic hormones on central neurons is of special interest in view of the fact that the secretion from the suprarenals may be increased under certain conditions. In asphyxia, a considerable increase has thus been observed (RAPELA and HOUSSAY 1952 a), and it is obvious that this finding should be taken into consideration in the inter-

pretation of frequency changes seen in spinal interneurons (KOLMODIN and SKOGLUND 1959) as well as in cortical neurons (CREUTZFELDT *et al.* 1957) during asphyxiation. Knowledge of the action of sympathetic amines *per se* on interneurons is, in fact, also a necessary basis for the analysis of the central action of certain drugs, such as nicotine, which is known to cause a greatly augmented adrenal secretion (RAPELA and HOUSSAY 1952 b, FOLKOW and EULER 1954).

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Renal Clearance in Dogs with Regard to Variations According to Age and Sex

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Abstract

Åsheim, Å., F. Persson and S. Persson, *Renal clearance in dogs with regard to variations according to age and sex.* Acta physiol. scand. 1961. *51.* 150—162. — Repeated determinations of renal clearance of inulin and *PAH* were done in 32 dogs. Statistical analysis showed no variations with age and sex. Repeated determinations of Tm_{PAH} were done in 16 dogs. The results indicated that even under standardized conditions Tm_{PAH} is highly varying. Statistical data are presented to show that the so-called self-depression begins to occur even at the lowest plasma-*PAH*-concentrations considered necessary for achieving a sufficient tubular load.

In most of the published investigations on renal clearance in dogs the normal values given are based upon small series of observations. There are only a few reports on statistically treated results of studies on large series. Houck (1948) presents data on glomerular filtration (GF) and effective renal plasma flow (RPF) in 75 normal dogs. Kubicek *et al.* (1953) reports similar results for 32 dogs. Kolberg (1959) quotes mean values for GF, RPF, and Tm_{PAH} (maximal tubular excretion capacity for para-amino-hippuric acid) in 11 dogs. All the said authors used female dogs. In the available literature only one publication is found in which male dogs were used in clearance studies, namely that by Stamler, Katz and Rodbard (1949). They report experiments with six male dogs and give the mean value for GF and RPF, but no *Tm*-determinations were done.

In all the above-mentioned studies, excepting those of Kolberg, only creatinine was used for measuring glomerular filtration rate. Kolberg gives results of inulin clearance as well.

As regards the determination of Tm_{PAH} in dogs, published data are very sparse. SMITH (1951) gives mean values for Tm_{PAH} calculated from results in various reports on small series of observations. KOLBERG (1959) gives the mean value for 11 dogs. No reports concerning Tm_{PAH} in male dogs can be found in the literature.

In the above-mentioned results of Tm_{PAH}-determinations the so-called self-depression was not taken into consideration, and, therefore, the quoted mean values may be suspected to be impaired by serious errors. This will be discussed later.

By means of a clearance technique, earlier authors have shown that renal function in children is fully developed at a very early age (WEST, SMITH and CHASIS 1948). Similar studies in young dogs have not been published.

In the work presented here normal values for GF and RPF, measured by inulin-clearance and *PAH*-clearance are given. In the statistical analysis variations in age and sex were taken into consideration. Determinations of Tm_{PAH} were also done and the value of such determinations in dogs is discussed.

Material

The experimental series consisted of 32 normal dogs, 21 females and 11 males. Out of these, 16 were mongrels and 16 cocker spaniels from five different litters. The latter were presumed to have an inherited disposition to hypoplasia of the renal cortex. Since the symptoms of this disease usually appear before the age of 1 $^1/_2$ year, renal function was studied continuously over this period of time. Only dogs without manifestations of the disease were included in this material. Besides clearance determinations, periodical checkings were done, which as regards the urine included specific gravity, protein, glucose, bilirubin, haemoglobin and sediment. The blood examinations comprised red-cell count, total white-cell count, erythrocyte-sedimentation rate, haemoglobin estimation, haematocrit, and non-protein nitrogen determination. The serum-level of electrolytes and cholesterol was also followed. ECG, intravenous urography and measurement of the intra-arterial pressure were carried out repeatedly. Eleven of these 16 dogs were subjected to autopsy after killing; the post-mortem examinations gave negative results. From another experimental series further normal values were calculated, but these will not be reported here so as to avoid heterogeneity.

Methods

Clearance determinations

Before each clearance observation the dogs were fasted for about 20 hours. This was done to obviate those variations in GF and RPF which are considered to occur at intake of protein. In the first few experiments 500 ml of water were given by the conventional method orally $^1/_2$ hour before the start of the observation to ensure satisfactory diuresis. The result of this was, however, that urinary output fell during the course of the experiment, and, hence, the influence of the "dead space" in the kidney was likely to increase. The clearance-values from these first experiments were, however, in full agreement with those obtained later, when the dogs were allowed water *ad lib.* Diuresis thus became even, and together with steady blood-levels of inulin and *PAH* the experimental conditions may be considered more standardized. Diuresis was as a rule moderate and was

never below 0.10 ml per minute. It should be noted here that the experiments included small dogs, too. Body-weight ranged from 7 to 16 kg.

The dogs were anesthetized with mebumal[1] given intravenously and since the observations were done in undisturbed and quiet surroundings, they could be kept in very light sleep right through the experiment. A Maghill's tube was used for intubation. A polyethylene catheter of conventional type was inserted into the cephalic vein and into the femoral artery (HELANDER, ÅSHEIM and ÖDMAN 1958). The vein catheter was used for giving the infusion, and the one in the artery for withdrawing specimens of blood and also for measuring the blood-pressure by connecting it with a mercury manometer. The initial dose of *PAH* and inulin was given, approximately 15 mg and 40 mg per kg body-weight, respectively. Continuous infusion of *PAH* and inulin was then started immediately by means of an electrically driven infusion apparatus. The concentration of *PAH* in blood plasma was maintained as close to 2 mg per 100 ml as possible, and the concentration of inulin between 15 and 30 mg per 100 ml. After a 45 min interval of equilibration the first clearance period was begun after the bladder had been emptied through a Foley catheter of suitable size. The evacuation of the bladder was aided by manual pressure on the abdomen over the bladder and by rinsing with physiological saline solution and a final rinsing with air. The urine was collected over a 20 min period. Blood was withdrawn from the femoral artery in the middle of the period. The heparinized centrifuge tube with the blood was immediately placed in a container with ice-water, where it was allowed to stand for about 5 min before centrifuging. Three such consecutive 20 min periods were completed. *Tm*-determinations were then made immediately. A primary dose of about 200 mg of *PAH* per kg body-weight was given by very slow injection. Continuous infusion of *PAH* and inulin was started. As far as possible a plasma-level of *PAH* exceeding 15 mg per 100 ml was maintained. The concentration of inulin was kept at the same level as earlier. After a 30 min equilibration interval two 20 min periods were as a rule completed.

Analytical methods for determination of the clearance agents

JOSEPHSON and GODIN's (1943) modification of CORCORAN and PAGE's (1939) method was used in the inulin analyses. *PAH* was determined by the method of SMITH (1951). All the analyses were carried out with a Beckman spectrophotometer, model B. Duplicate determinations were made of the inulin and *PAH* concentrations in each specimen of blood and urine and the means were calculated.

Results

A. Glomerular filtration rate and effective renal plasma flow

The results for the 16 cocker spaniels are given in Table I. The total number of clearances is 54, with approximately 162 observations. The values for both GF and RPF are expressed per kg body-weight and per sq. m body surface (COWGILL and DRABKIN 1927).

[1] The formula of mebumal is as follows:

5-ethyl-5(1-methylbutyl)-malonylcarbamide (Pentobarbitone)	1.8 g
Pentobarbitone sodium	4.0 g
Urethane	25.0 g
Spir. conc.	15.0 g
Glycerin	12.5 g
Aq. steril.	ad 100 ml

Table I. Clearance of inulin and PAH in 16 cocker spaniels. Each value is the average of 1 to 4 observations, each of which in turn is the average of 3 consecutive periods

Dog	Sex	Filtration rate (C_{In}) ml/min.		Renal plasma flow (C_{PAH}) ml/min.		Filtration Fraction C_{In}/C_{PAH}
		per kg	per m²	per kg	per m²	
I: 2	♀	4.33	89	15.85	325	0.28
I: 3	♀	3.92	88	11.98	270	0.30
I: 4	♀	3.18	66	10.47	218	0.31
II: 1	♀	4.49	96	12.37	267	0.36
II: 2	♀	3.64	74	16.91	350	0.24
II: 3	♀	3.05	67	10.88	242	0.28
II: 5	♂	3.88	91	12.63	301	0.30
III: 1	♂	4.16	90	12.24	324	0.28
IV: 1	♀	3.12	61	12.67	249	0.26
IV: 2	♀	3.59	72	14.34	288	0.28
IV: 3	♂	4.21	91	17.83	389	0.24
V: 2	♂	3.71	80	13.71	294	0.27
V: 3	♀	3.84	70	15.77	288	0.25
V: 4	♂	5.86	100	17.71	301	0.33
V: 5	♂	2.94	54	9.03	167	0.32
V: 6	♀	3.47	62	14.68	258	0.38

Since the values in Table I represent observations on dogs of various ages and of both sexes, it was considered necessary to study any presence of significant variation of the values with age and sex before treating the whole material statistically. The results from the clearance tests arranged in age-groups, showed no tendency to variation with age within the range concerned.

For calculation of any variation with sex the *t*-test was used. The mean values for C_{In} and C_{PAH} calculated from values given in Table I showed no significant differences.

Both for C_{PAH} and for C_{In} $P > 0.05$. The *t*-value for C_{In}, however, is as great as 1.177.

Since glomerular filtration is reported to be higher in men than in women, and since our material comprises only 6 male dogs, the series was augmented by 16 mongrel dogs, including 6 males. The age of these 16 dogs corresponded to that of the rest of the series. The values for this additional material are given in Table II, arranged as Table I.

In consideration of this larger material ($n = 32$) (Table I and Table II) with respect to sex difference, it will be seen that there is no significant deviation either for C_{In} ($0.3 > P > 0.2$) or for C_{PAH} ($0.2 > P > 0.1$). The mean values for C_{In} are for male dogs 81 ± 4.9 ($n = 11$) and for female dogs 71 ± 3.1 ($n = 21$). The mean values for C_{PAH} are for male dogs 286 ± 24.3 ($n = 11$) and for female dogs 251 ± 10.5 ($n = 21$).

Table II. Clearance values from 16 mongrel dogs

Dog	Sex	Filtration rate (C_{In}) ml/min.		Renal plasma flow (C_{PAH}) ml/min.		Filtration fraction C_{In}/C_{PAH}
		per kg	per m²	per kg	per m²	
1	♂	2.72	63	9.44	220	0.29
2	♂	3.50	75	11.00	236	0.32
H: 2	♂	4.66	102	14.60	321	0.32
H: 3	♀	4.43	75	11.29	193	0.38
H: 5	♀	3.50	67	10.20	196	0.35
H: 6	♀	4.10	79	13.10	251	0.31
H: 7	♀	4.71	80	10.86	185	0.44
H: 8	♀	4.33	80	11.67	216	0.38
H: 9	♂	4.36	86	21.18	419	0.26
H: 10	♀	4.70	90	14.70	282	0.32
H: 11	♀	4.57	78	17.00	289	0.26
H: 26	♂	2.64	61	7.64	175	0.34
H: Gr	♀	1.74	44	6.30	160	0.27
H: St	♀	3.11	71	9.88	226	0.32
H: Sc	♀	4.11	94	12.82	293	0.32
H: Co	♀	2.20	42	11.30	217	0.19

Since there seems to be no significant variations with repect to sex or age, the data concerning C_{In}, C_{PAH} and FF (filtration fraction, C_{In}/C_{PAH}) for the whole material have been analysed statistically and the results appear in Table III.

Skewness of the distribution was estimated by Lindeberg's formula:

$$S_L = P - 50,$$

where P is the percentage of the total number of observations whose value exceeds the mean. The standard error ΣS_L for S_L was calculated from the formula:

$$\Sigma S_L = \pm \frac{30}{\sqrt{n}}$$

and with Student's t-test the following was obtained:

$S_L \pm \Sigma S_L$ for:

C_{In}/kg : 3.12 ± 5.30	$(0.6 > P > 0.5)$
C_{In}/m² : 0 ± 5.30	$(P = 1)$
C_{PAH}/kg : -9.38 ± 5.30	$(0.1 > P > 0.05)$
C_{PAH}/m² : 3.13 ± 5.30	$(0.6 > P > 0.5)$
FF : -3.12 ± 5.30	$(0.6 > P > 0.5)$

It will be seen from these calculations that the distributions can be regarded as normal.

Table III. Statistical analysis of filtration rate and effective renal plasma flow in normal dogs

	C_{In} ml/min.		C_{PAH} ml/min.		Filtration fraction C_{In}/C_{PAH}
	per kg	per m²	per kg	per m²	
	No. of dogs 32				
Mean	3.77	76	12.88	263	0.31
Range	1.74–5.86	42–102	6.30–21.18	160–419	0.19–0.44
Standard deviation	0.84	15	3.21	62	0.051
Standard error	0.15	2.69	0.57	11.17	0.0089
Coefficient of variation	22.2	19.8	24.9	23.6	16.5

B. Maximal tubular excretion capacity for PAH

By subtracting the amount of *PAH* which is filtered through the glomeruli from the amount excreted in the urine per unit of time a measure is obtained of the amount of *PAH* actively excreted via the tubules. This can also be expressed by the formula (SMITH 1951):

$$T_{PAH} = U_{PAH} \times V - C_{In} P_{PAH} FW$$

where the symbols denote conventional values. In dogs *FW* is considered to be constant and independent of the plasma concentration up to 60 mg per 100 ml and is quoted at 0.917 (SMITH 1951). In our calculations the constant 0.92 was used throughout. The results of the T_{PAH}-determinations appear in Table IV. Four negative values from two dogs are listed separately in Table V. The accumulation of *PAH* in the tubules is calculated from the formula:

$$P_{PAH} \times C_{PAH} - C_{In} \times P_{PAH} \times FW$$

each expression being of conventional denotation.

Since there is great variation in the T-values and this variation seems to show some correlation with the blood-plasma concentration of *PAH*, the values were inserted in a co-ordinate system with T_{PAH} as the ordinate and P_{PAH} and the ratio load/T_{PAH}, respectively, as the abscissa (Fig. 1 and 2). It will be seen from these two figures that there is a tendency to falling T_{PAH} with rising P_{PAH} or rising load/T_{PAH}. The values given in Fig. 1 were treated statistically by regression analysis (Fig. 3). To find out whether the regression was linear the material was grouped in such a way that P_{PAH} was given in a precision of 5 mg/100 ml. The T-variable is called y and the designation x is used for the integral part of $(PAH - 10)/5$. The regression analysis gives the straight line $y = 25.68 - 0.70\,x$ and the standard deviation around the line 8.91. The present material does not show a significant deviation from a linear regression, that is, a linear regression is accepted. A test for significance shows that the regression coefficient diverges significantly from 0, that is, a hypothesis of a negatively inclined straight regression line is accepted.

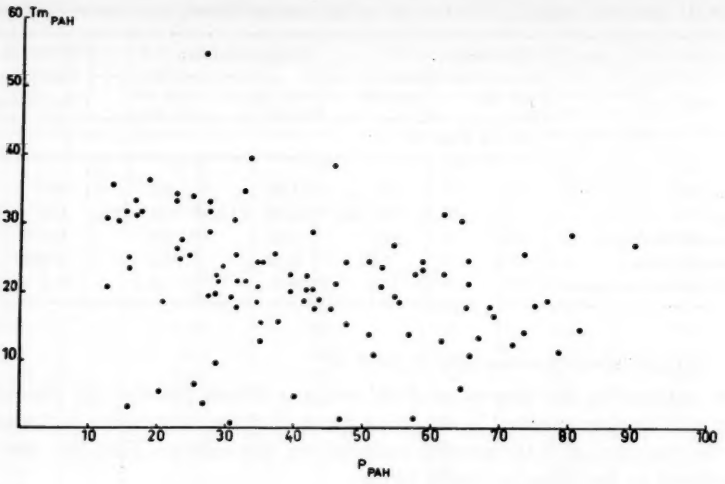

Fig. 1. One hundred Tm_{PAH}-determinations with various plasma-concentrations (P_{PAH}). Tm_{PAH} is given in mg/min/m² and P_{PAH} in mg/100 ml.

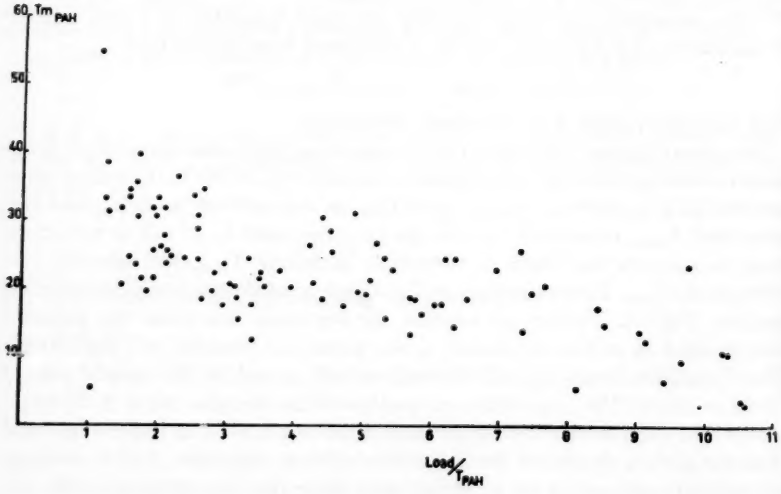

Fig. 2. The same Tm_{PAH}-determinations as in fig. 1, now in regard to the ratio load/T_{PAH}. The six lowest values for Tm_{PAH} (highest load/T_{PAH}) are not included. Tm_{PAH} is given in mg/min/m².

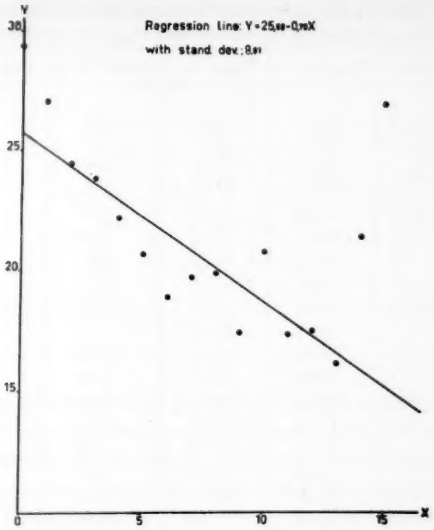

Fig. 3. Regression of y on x, where y is Tm, given in mg/min/m² and x is the integral part of $\frac{PAH - 10}{5}$. P_{PAH} is given in mg/100 ml.

To enable a comparison between our results and those given by different authors we have calculated T_{PAH} for those values which were obtained at a tubular load/T_{PAH} between 1.5 and 4.0. For 46 such observations the mean value for T_{PAH} is 25.43 with standard deviation 7.04 mg per minute per sq. m, or 1.21 with the standard deviation 0.34 mg/min/kg.

Table V records the afore-mentioned negative T-values. The possibility that these are due to some analytical error cannot be excluded, although there is nothing to suggest such an error. The occurrence of negative T-values would mean that some part of the filtrated *PAH* as well is reabsorbed. Schachter and Freinkel's theory that self depression would be due to reversible interferences with the mechanism of transport in the tubules is, however, supported by MUDGE and TAGGART (1950) who by administration of acetate were able to control this self-depression and raise the maximal tubular excretion. Since the T_{PAH}-determinations reported here were done in cocker spaniels, the question arises as to whether this breed reacts in a specific way. In a series of 16 dogs of various breeds, however, the results of 23 Tm-determinations at various plasma levels of *PAH* were analogous to those reported for cocker spaniels.

Discussion

Anesthetized dogs were used in the studies. Deep anesthesia for a prolonged period may probably depress certain bodily functions, such as temperature, blood-pressure, and, as a result of the altered blood pressure, renal function as well.

Table IV. T_{PAH} determinations in correlation to plasma-concentration (P_{PAH}) and ratio load/T_{PAH}. T_{PAH} is given as mg/min/m², and P_{PAH} as mg/100 ml. Number of observations = 100

Dog	T_{PAH}	P_{PAH}	Load/T_{PAH}	Dog	T_{PAH}	P_{PAH}	Load/T_{PAH}
I: 2	33.81	25.00	2.50	III: 1	9.70	28.41	9.63
	30.44	14.17	1.67		6.59	25.89	9.42
	13.86	50.94	9.01		25.21	31.37	4.54
	18.84	43.42	5.76		19.93	28.39	4.90
	17.47	45.09	4.71		33.16	22.55	1.56
	24.05	35.07	2.59		31.29	16.70	1.24
	31.02	61.79	4.87		34.25	22.55	1.55
	20.89	52.61	7.53		33.24	16.67	1.20
I: 3	39.66	33.33	1.68	IV: 1	17.98	68.47	14.89
	24.64	23.33	1.56		23.22	58.45	9.75
	18.80	41.67	3.13		18.92	76.82	5.67
	15.64	35.00	3.16		20.98	65.13	4.26
	20.15	42.51	3.59		14.05	73.48	6.31
	17.64	31.67	2.95		13.24	66.80	5.69
	19.20	54.28	7.44		17.73	42.59	3.37
	15.08	47.60	8.40		19.92	39.25	2.77
I: 4	28.89	42.50	3.03	IV: 2	30.69	30.90	2.56
	31.88	27.50	1.88		27.83	23.38	2.04
	5.89	64.30	1.15		14.60	81.80	13.63
	1.38	57.78	42.01		11.41	78.50	17.67
	30.50	64.30	4.39		24.38	34.40	2.37
	26.59	54.28	4.10		23.87	29.23	2.09
	26.86	89.35	5.19		15.87	37.58	5.30
	28.15	80.16	4.50		21.63	32.52	3.46
II: 1	38.55	45.65	1.12	IV: 3	17.95	75.00	6.02
	12.81	34.86	3.37		16.15	69.00	5.84
	18.54	55.11	6.50		35.72	13.36	1.64
	10.96	51.77	10.25		30.71	12.53	1.93
	25.51	73.48	7.28		36.18	18.37	2.24
	22.96	61.79	6.93		31.89	15.03	2.07
II: 2	22.88	57.50	5.41	V: 2	23.40	15.87	1.63
	1.49	46.67	56.02		20.54	12.53	1.42
	21.07	45.93	5.08	V: 3	22.03	41.75	3.46
	4.59	40.08	17.51		20.46	34.24	3.08
	19.17	30.90	5.01		34.77	32.57	2.61
	24.80	15.87	2.17		28.87	27.56	2.57
	24.34	50.90	6.54		5.37	20.04	8.11
II: 3	17.97	65.00	6.15		3.07	15.87	10.58
	12.99	61.66	8.52	V: 4	54.74	26.72	1.13
	13.91	56.78	7.33		31.87	17.54	1.41
	20.11	41.72	3.91	V: 5	24.33	65.13	3.25
	12.21	71.81	9.19		23.71	52.61	2.98
	10.62	65.97	10.30		21.59	31.73	1.90
	22.53	39.25	3.97		19.39	27.59	1.80
	22.32	28.39	2.80	V: 6	24.19	58.45	6.18
II: 5	18.52	34.33	4.38		24.09	47.60	5.29
	25.50	24.22	1.91		25.55	36.74	2.11
	18.57	20.88	2.61		21.72	29.23	1.73
	33.15	27.56	1.96		0.74	30.90	53.53
	26.13	22.54	2.00		3.77	26.72	10.55

Table V. Clearance values in 2 dogs, each with 2 observations which gave negative T_{PAH}

Dog	II: 1		V: 5	
Weight kg	11	11	11	11
Body surface area m^2	0.524	0.524	0.524	0.524
Period (minutes)	1(20)	2(20)	1(15)	2(15)
Diuresis ml/min.	0.55	0.43	0.33	0.33
Urine concentration of *PAH* mg/100 ml	6,050	7,050	3,000	2,550
Plasma concentration of *PAH* mg/100 ml	75.15	63.46	35.07	28.39
Filtration rate C_{In} ml/min.	75	86	37	35
Renal plasma flow C_{PAH} ml/min. Mean value	157	157	79	79
T_{PAH} mg/min.	−18.6	−19.9	−2.0	−0.7

CORCORAN and PAGE (1943) state that anesthesia induced by pentobarbitone sodium does not influence the clearance of inulin and diodrast, but that a slight rise of the arterial pressure would occur. CRAIG, VISSCHER and HOUCK (1945) have studied renal function in the dog under ether and cyclopropane anesthesia. With light anesthesia renal function is not affected, while under deep anesthesia it is reversibly depressed. REMINGTON *et al.* (1949) have shown that barbiturates given i.v. produce first hypotension and then slight hypertension. SELKURT and GLAUSER (1951) consider that the reabsorption of sodium is slowed down under prolonged surgical anesthesia induced by pentobarbitone sodium.

The effect of anesthesia on renal function is thus dependent upon the depth of anesthesia. In the present experiments mebumal was used as an anesthetic. Very light sleep was induced and its depth was checked by the eye-closure and toe reflexes. Such checking will, of course, allow some subjectivity. A fact that lends support to the presumption that the anesthesia did not depress renal function is that the clearance observations were not continued for more than two hours, while in the *Tm*-determinations, which were always done immediately after the clearance tests, the dogs were kept anesthetized for about 3 hours. GLAUSER and SELKURT (1952) state that in deep and prolonged anesthesia barbiturates do not affect glomerular filtration but reduce total plasma flow. Accordingly, with such an effect FF should increase. In our experiments the filtration fraction was throughout very constant during the different clearance-periods, and, therefore, no influence whatsoever can have interfered with the determinations of C_{In} and C_{PAH}.

GLAUSER and SELKURT have shown that barbiturate anesthesia not exceeding 3 hours does not involve any risk of depressed renal function. On the other hand, WHITE (1957) and STÖREN (1958 a and b) have demonstrated a lowering of Tm_{PAH} under anesthesia induced by pentobarbitone and thiopentone sodium. The Tm_{PAH} values given by STÖREN were, however, even for unanesthetized

dogs lower than those obtained by us. In our *Tm*-determinations the anesthesia lasted for about 3 hours and was very light.

As regards the arterial blood pressure we found full agreement with the studies by REMINGTON *et al.* (1949). As has been reported in an earlier paper (ÅSHEIM, HELANDER and PERSSON 1958), the blood pressure was on several occasions also measured before the induction of anesthesia was started. No significant differences between blood pressure values before and during the anesthesia were noted.

One disadvantage of mebumal is that it contains alcohol which causes a tendency to hemolysis. To diminish hemolysis the collected blood specimens were treated in a special way, as described previously.

The values for C_{In} and C_{PAH} obtained from our observations are in good agreement with values reported earlier by HOUCK (1948) and KUBICEK *et al.* (1953), among others.

Concerning *Tm*-variations in dogs the literature contains very sparse statistical data with varying results (MUDGE and TAGGART 1950, SMITH 1951, KOLBERG 1959). The results of our studies disaffirm the occurrence of truly constant Tm_{PAH}-values in dogs, even though the plasma level of *PAH* is kept within accepted limits. Instead, it will be seen that T_{PAH} approaches *0* as P_{PAH} rises. This phenomenon, in the literature referred to as self-depression, has been discussed by several authors. In cats EGGLETON and HABIB (1950) were the first to show such a depression of Tm_{PAH} at plasma-*PAH* concentrations exceeding 30 mg per 100 ml. They considered that the depression was due either to a back-diffusion in the tubules of *PAH* or to a toxic effect on the proximal tubular cells. The value of their study is somewhat lessened by the fact that their observations were made at rapidly changing plasma concentrations, which is a well documented cause of misjudgements. JOSEPHSON *et al.* (1953) have confirmed EGGLETON and HABIB's results by showing that in the cat there is a complete inhibition of the tubular excretion at plasma-concentrations of about 20 mg of *PAH* per 100 ml or 30 mg of Diodon per 100 ml. They also found negative *T*-values, which was considered to suggest a reabsorption of *PAH* and Diodon. In similar experiments in rabbits inhibition of tubular excretion was not noted. SCHACHTER and FREINKEL (1951) have demonstrated self-depression for Tm_{PAH} in non-anesthetized dogs. They hold that self-depression can be seen or fail to occur on different occasions in the same animal. They assume that the self-depression is due to reversible interferences with the tubular transport and not to back-diffusion or to toxic effect. They consider that self-depression occurs at a ratio of load/T_{PAH} that is higher than 6 and that the definite Tm_{PAH} should be calculated from values obtained at load/T_{PAH} between 1.5 and 4.0. STÖREN (1958 a), in a recently published study on Tm_{PAH} in dogs, has demonstrated that self-depression occurs earlier (load/$T = 4$) under pentobarbitone anesthesia than in nonanesthetized dogs.

BARCLAY, COOKE and DE MURALT (1950) have noted reabsorption of *PAH*

and Diodon from normal kidneys in man. Negative Tm-values in renal disease both in man and in dogs have also been reported by some authors, among others JOSEPHSON (1947). JOSEPHSON points out that EGGLETON and HABIB's results in cats can hardly be relevant to man, since such high blood-concentrations are not used for studies on man. There is no conclusive evidence of the occurrence of self-depression in man (JOSEPHSON 1947, JOSEPHSON *et al.* 1952, JOSEPHSON and EK 1958).

POULSEN (1956), in studies on cows, has observed a lowering of Tm for Diodrast, when the tubular load is increased to such an extent that the ratio load/T_D exceeds 5.

The results obtained in our studies of Tm_{PAH} indicate that the maximal active tubular excretion in dogs under the standardized experimental conditions is highly varying. We have thus confirmed SCHACHTER and FREINKEL's observations concerning self-depression. In opposition to these authors, however, we have shown that a constant Tm_{PAH} cannot be obtained even at a certain maximal plasma-concentration of *PAH*. Self-depression begins to occur even at the lowest *PAH* concentrations considered necessary for achieving a sufficient tubular load.

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Acta physiol. scand. 1961. 51. 163—168

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Electromyographic Findings in Experimental Botulinum Intoxication

By

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Abstract

Josefsson, J.-O. and S. Thesleff. *Electromyographic findings in experimental botulinum intoxication.* Acta physiol. scand. 1961. *51.* 163—168. — The electrical activity of botulinum intoxicated skeletal muscle was examined in the rabbit by the use of electromyography. 5 to 6 days after an intramuscular injection of the toxin, fibrillation potentials appeared and persisted for a period of at least 60 days. The potentials were similar in amplitude and time course to those recorded following motor nerve degeneration. Botulinum toxin, however, is known not to affect the morphological structure of the motor nerve and its terminals. Its sole action is to block transmitter release selectively from cholinergic nerve endings. Our results therefore suggest that in nerve degeneration lack of transmitter release is primarily responsible for initiating the process which produces the electromyographic pattern typical of denervation.

Degeneration of a motor nerve is generally not associated with complete cessation of the electrical activity of muscle fibres. For a period after denervation, the length of which varies from species to species, there is no activity. But afterwards there occur spontaneous action potentials of short duration and low voltage (0.5—3 msec and 50—300 μV). These potentials are thought to arise from single muscle fibres, and are called fibrillation potentials. Generally, the mechanical activity produced by fibrillations is too feeble and asynchronous to be visible.

The fibrillation activity of chronically denervated muscles is likely to be due to the absence of some influence exerted when the motor innervation is intact. The purpose of the present investigation has been to determine whether or not the release of the chemical transmitter agent provided such an influence. Use

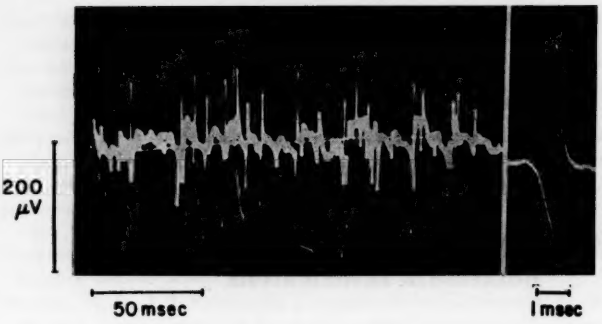

Fig. 1. Fibrillation potentials in the rabbit tibialis ant. muscle 20 days after the administration of botulinum toxin. The record on the right shows one of these potentials at a faster sweep speed.

was made of botulinum toxin, which has been shown to prevent the release of acetylcholine (ACh) from cholinergic nerves without causing degeneration of the axis cylinder or the nerve terminals. (AMBACHE 1949, BURGEN, DICKENS and ZATMAN 1949, BROOKS 1956, THESLEFF 1960). It will be shown that in botulinum intoxicated muscles there occur fibrillation potentials similar to those in denervated muscles, and that, consequently, in nerve degeneration lack of transmitter release is to be considered responsible for the spontaneous activity of denervated muscles.

Methods

A powdered preparation of Cl. botulinum toxin type A with a mouse LD_{50} of 0.05 μg/kg was used. 1 mg of the toxin was dissolved in 1 ml of sterile phosphate buffer as described by AMBACHE (1949). Further dilutions of the toxin were made from this stock solution immediately before use. A fresh stock solution was prepared for each day's experiment.

The toxin, in amounts ranging from 0.06 to 0.1 μg was injected in divided amounts into the musculature of the hind legs of 22 rabbits. With those doses and mode of administration the action of the toxin was confined mainly to the site of application. Generalized intoxications were only observed with the highest dose of the toxin. As described by GUYTON and MACDONALD (1947) and THESLEFF (1960) the neuromuscular block produced by a single dose of botulinum toxin reaches its maximum in about 5 days, and then remains at a constant level for a period of several months.

For comparison the sciatic nerve was cut in 20 rabbits, producing complete motor denervation of the hind legs. At two day intervals for a period between 3 and 8 weeks after the administration of the toxin, or the denervation procedure, the electrical activity was recorded in the hind legs by the use of electromyography.

Electromyographic recording was made by a concentric needle electrode (Disa, 250 K Ω shunted by 15 nF at 100 c/sec). The electrode was connected through an amplifier (Grass model P 5) to a cathode-ray oscilloscope. The potentials were photographed on single successive sweeps. The amplitude of individual potentials was taken as their peak-to-peak value. The duration was the time interval between the initial deflection from the base line and the point at which the terminal deflection again returned to the base line.

In order to study the response of botulinum intoxicated muscles to ACh, a cannula was introduced into the iliac artery. Through this cannula intra-arterial injections of

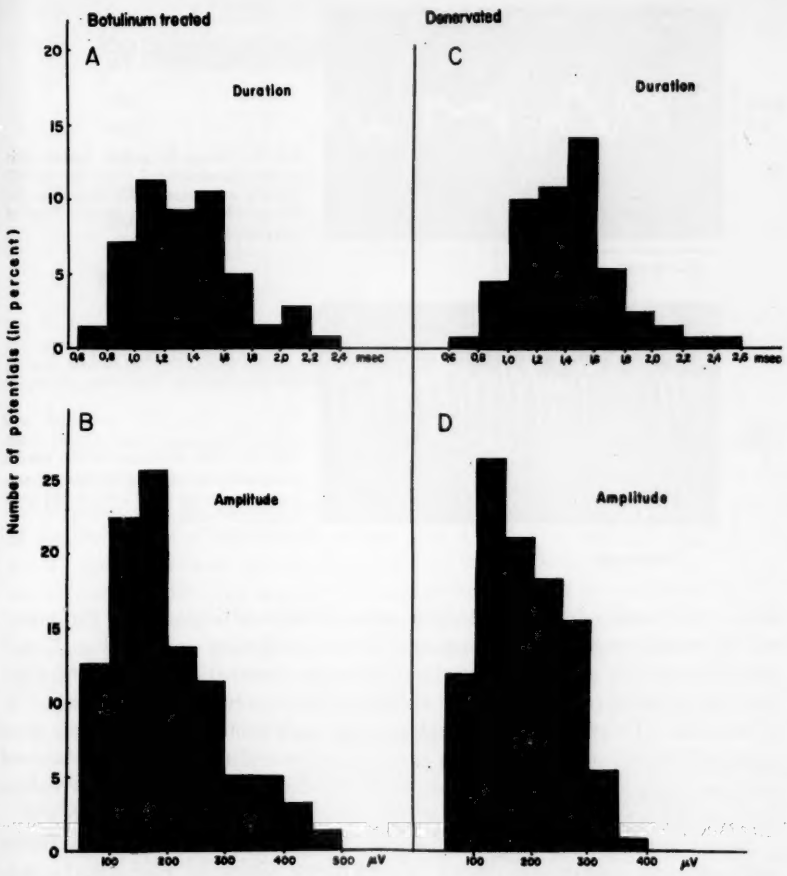

Fig. 2. Distribution of durations and voltages of potentials of the fibrillation type recorded in the tibialis ant. muscle of the rabbit 10 to 30 days after botulinum administration (A and B) or motor denervation (C and D). The graphs A and C were derived from 70 potentials; B, from 160; D, from 109.

ACh were made in a volume of 0.5 ml. Electromyographic records of the response of the tibialis anterior muscle to the injection of the drug were made simultaneously with a record of its isometric tension (registered on a Grass model 5 polygraph).

Results

About 5 days after the intramuscular administration of botulinum toxin occasional action potentials of low voltage and short duration appeared. At the same time a desynchronization and a reduction of duration and amplitude of

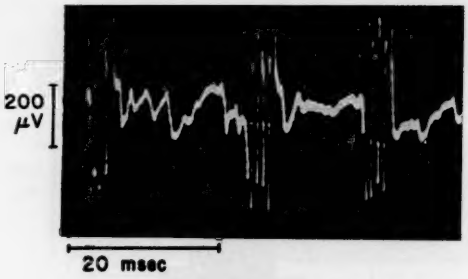

Fig. 3. Desynchronized motor unit potentials recorded from the rabbit tibialis ant. muscle 18 days after the administration of a small dose of botulinum toxin.

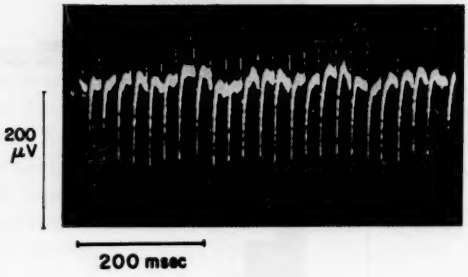

Fig. 4. Repetitive potential discharge following mechanical stimulation (the insertion of the needle electrode) in the rabbit tibialis ant. muscle 17 days after botulinum toxin administration.

motor unit potentials occurred. After about 10 days of intoxication the motor unit potentials were replaced by potentials of the fibrillation type (Fig. 1) and they subsequently persisted throughout the entire observation period of 60 days. The distributions of durations and voltages of such potentials are given in Fig. 2 A and B. (To exclude the possibility that small motor unit potentials were included, the sciatic nerve was cut prior to the recording). It may be observed that the duration of the potentials in botulinum intoxicated muscles ranges between 0.6 and 2.4 msec, and that their mean amplitude is about 195 μV.

In instances of slight intoxication small and frequently desynchronized motor unit potentials were present (Fig. 3). Furthermore, following mechanical stimulation, bursts of single potential discharges with a slowly diminishing frequency were recorded (Fig. 4).

Animals which did not receive botulinum toxin, but were denervated by cutting the sciatic nerve, showed typical denervation fibrillations 4 to 5 days after the operation. The distributions of durations and amplitude of such fibrillation potentials are shown in Fig. 2 C and D.

Their mean duration and amplitude were 1.4 msec and 184 μV, which closely correspond to the 1.3 msec and 195 μV values which are the mean of similar potentials in botulinum intoxicated muscles.

Brown (1937) and, subsequently, Rosenblueth and Luco (1937) have shown that close arterial injection of ACh in a chronically denervated mammalian muscle produces an electrically silent contracture, after an initial burst

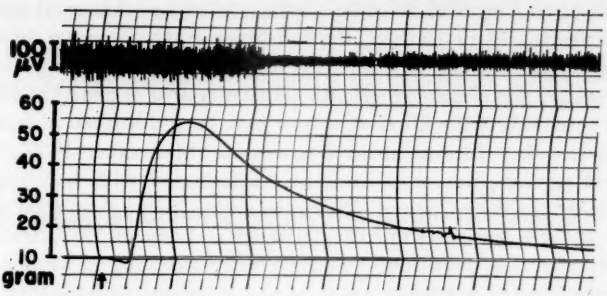

Fig. 5. The response of the tibialis ant. muscle to intra-arterial injection of ACh 16 days after the administration of botulinum toxin. The upper tracing shows the electrical activity of the muscle and in the lower tracing its isometric tension is recorded. The arrow shows the moment when 60 μg of ACh was injected into the iliac artery. Time scale, one sec/square.

of twitches. In an innervated muscle ACh caused muscle twitches but never a lasting increase in tension. It was therefore of interest to examine whether or not the response of a muscle chronically intoxicated with botulinum to ACh would be similar to that of denervated muscle. Fig. 5 shows the response to ACh of a tibialis anterior muscle paralysed for 16 days by botulinum toxin. When given intra-arterially the drug produced a contracture, which was accompanied by a marked reduction of the electrical activity of the muscle. The effect of ACh was thus similar to that produced in muscles following degeneration of their motor nerve.

Discussion

Characteristic of botulinum poisoning is a lack of transmitter release from cholinergic nerves, while otherwise nerve and muscle are unaffected by the toxin (AMBACHE 1949, BURGEN et al. 1949, WRIGHT 1955, BROOKS 1956, STEVENSON 1958, LAMANNA 1959). Recently KATZ (quoted by THESLEFF 1960) has demonstrated that the ultrastructure of motor nerve terminals is unaltered by the toxin. Thus degenerative changes in the nerve endings similar to those described during Wallerian degeneration (BIRKS, KATZ and MILEDI 1960) are not observed even after long-lasting and complete paralysis produced by botulinum toxin. It is consequently reasonable to assume that the toxin has a selective mode of action, and that its site of action is at the mechanism responsible for transmitter release from cholinergic nerves (THESLEFF 1960).

As shown by the present investigation, the electromyographic findings in chronic botulinum poisoning are almost identical with those found following motor denervation. Typical and almost diagnostically significant for chronic denervation are fibrillation potentials (DENNY-BROWN and PENNYBACKER 1938). These potentials were observed in botulinum intoxicated muscles. Since, as

mentioned, botulinum toxin is without effect on the morphological structure of the motor nerve and its endings, the conclusion is that in nerve degeneration lack of transmitter release is, primarily responsible for initiating the process which produces the electromyographic pattern typical of denervation.

Action potentials of the fibrillation type have been described as occurring in fetal muscle before its functional innervation (MARINACCI 1959), in adult denervated muscles, and now in muscle intoxicated with botulinum. It is interesting that in those three conditions the entire muscle membrane also has a high and uniform sensitivity to ACh (DIAMOND and MILEDI 1959, AXELSSON and THESLEFF 1959, THESLEFF 1960). It is therefore tempting to assume that the spontaneous fibrillation activity is in some way correlated with the high chemical sensitivity of the muscle fibre, but such a connexion remains to be shown experimentally.

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Acta physiol. scand. 1961. 51. 169—174

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Cineangiocardiographic Studies of Puppies and Full-Grown Dogs

By

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Abstract

GRIBBE, P., L. HIRVONEN and T. PELTONEN. *Cineangiocardiographic studies of puppies and full-grown dogs.* Acta physiol. scand. 1961. *51.* 169—174. — Cineangiocardiographic studies have been carried out on puppies and full-grown dogs. The volumes of the left atrium and the left ventricle were calculated using 35-mm film, exposed at a rate of 48 pictures per second. The stroke volume and residual blood volume were calculated from the volume curves. The stroke volume calculated as a percentage of the end-diastolic volume was larger for the puppies (77 per cent) than for the full-grown dogs (60 per cent). In relation to their body weight the stroke volume for the full-grown animals was 60 per cent lower than that for the puppies. The pulmonary circulation time increased with age. It was shorter in puppies than in full-grown dogs, both absolutely and in terms of the number of cardiac cycles which it occupied. The prolongation of the pulmonary circulation time with advancing age, when expressed in terms of the number of heart cycles which it occupies, may be mainly due to a decrease of the stroke volume in relation to the body weight.

Cineangiocardiography is a useful method for studying cardiac dynamics. As shown in a previous paper (GRIBBE et al. 1959) it is possible to calculate the stroke and the residual blood volumes of the left ventricle, the cardiac output and the pulmonary circulation time from angiocardiograms, recorded at a high rate during the passage of blood containing radiopaque material through the heart cavities. In the present paper cineangiocardiographic findings in young and full-grown animals are compared.

Methods

We employed as our experimental animals 6 puppies, aged between 63 and 91 days and weighing 2.3—4.6 kg and 20 full-grown dogs weighing 7—19.5 kg.

Table I. Duration of various phases of the mechanical heart cycle in puppies and full-grown dogs

	Cycle length (msec)	Isometric contraction	
		msec	% of cycle
2—3-month puppies (9 experim.)	333 ± 13	48 ± 3	14,5 ± 0,7
Full-grown dogs (13 experim.)	420 ± 23	60 ± 3	14,6 ± 0,8

The animals were anesthetized with an intraperitoneal injection of Nembutal. The dose varied from 30 to 40 mg per kg of body weight.

A Cournand catheter or a polyethylene tube was introduced through the external jugular vein into the right heart. The injection of contrast medium (Urografin 76 %, Schering-Leiras, 1.4 ml per kg of body weight) was performed with a Gidlund automatic pressure syringe into the right atrium. The angiocardiograms were taken with a 5-inch Philips' image intensifier and an Arriflex 35-mm camera employing an exposure rate of 48 pictures per second. The right oblique anterior projection was used. Electrocardiograms were taken simultaneously employing synchronous picture marking.

The diameters of the left atrial and of the left ventricular cavities could be measured frame after frame from the levogram and their volumes could be calculated by the formula given by GRIBBE et al. (1959).

Results

The heart rate was slightly higher in the puppies (184/min.) than in the full-grown dogs (169/min.).

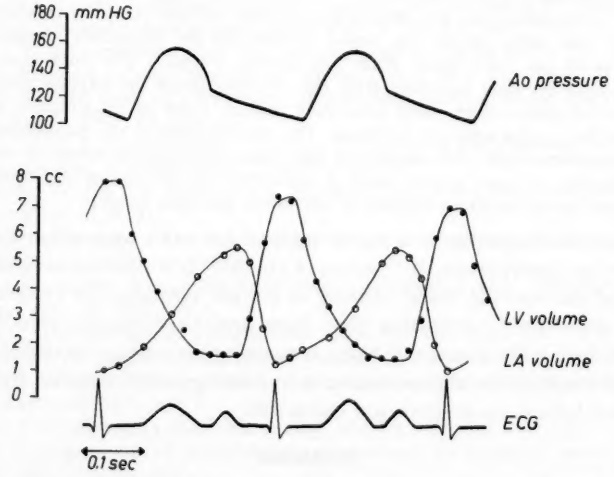

Fig. 1. Volume curves of the left heart of a puppy. Note the large stroke volume of the left ventricle in relation to the end-diastolic volume.

Means ± errors of the means

Ejection		Isometric relaxation		Filling	
msec	% of cycle	msec	% of cycle	msec	% of cycle
127 ± 7	38,4 ± 2,1	76 ± 5	23,0 ± 1,6	82 ± 9	24,1 ± 1.7
128 ± 12	30,6 ± 2,3	90 ± 5	22,3 ± 1,3	142 ± 18	32,5 ± 2,5

The various phases of the mechanical cardiac cycle can be calculated with sufficient accuracy by studying the films. The phases of the heart cycle are presented in Table I. The greatest difference is observed in the filling phase which is both absolutely and relatively longer in the full-grown dogs than in the puppies, 1.75 times as long in seconds and 1.45 times as long when computed as a fraction of the cardiac cycle. The isometric contraction and relaxation phases expressed in milliseconds were somewhat longer in the full-grown than in the puppies, but the ejection phase was of the same length in both groups.

Left ventricular volume curves for the two dog groups are shown in Fig. 1 and 2. The curves have been drawn in the correct time relationship to the simultaneously recorded electrocardiograms.

The results of the volume measurements are shown in Table II. The relative *end-diastolic volume* (in per mille of body weight) was slightly, but not significantly, larger in the puppies than in the full-grown dogs.

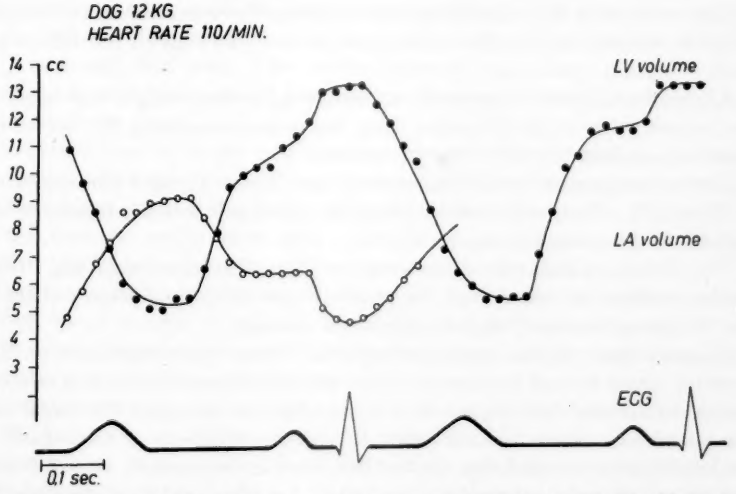

Fig. 2. Volume curves of the left heart of a full-grown dog. Note the length of the filling phase of the left ventricle.

Table II. Results of volume calculations of the left ventricle in various animal groups

The numbers in parentheses give the ranges

Animal group	End-diastolic volume		Residual blood volume (ml)	Stroke volume			Heart rate	Minute volume ml/kg b.w
	ml	‰ of b.w.		ml	‰ of b.w.	% of end-diastolic volume		
2–3-month-old puppies	4.9 (3.1–7.4)	1.59±0.13	1.1 (0.6–1.6)	3.8 (2.3–5.8)	1.23±0.10	76.7±1.0	184±7	225 (152–313)
Full-grown dogs	12.9 (5.9–25.5)	1.25±0.07	5.1 (3.1–10.0)	7.8 (3.9–15.5)	0.75±0.04	60.4±0.7	169±5	124 (79–151)

The stroke volume relative to the body weight was 1.23 per mille of body weight for the puppies. A comparison of the stroke volume of the puppies and full-grown dogs (0.75 per mille) revealed a significant decrease (difference 0.48 ± 0.11 per mille) with increasing age.

The stroke volume expressed as a percentage of the end-diastolic volume decreased clearly with age. The difference in mean stroke volume is significant between the puppies and full-grown dogs. The stroke volume was 77 per cent of the end-diastolic volume in the puppies and 60 per cent in the full-grown dogs; the difference is 17 ± 1 per cent.

This means that *the residual blood volume* expressed as a percentage of the end-diastolic volume was smaller in the younger animals than in the full-grown ones.

As seen from Table II, *the minute volume* per kg of body weight was larger in the puppies than in the full-grown dogs, the mean value being 225 ml for the former group and 124 ml for the latter group.

The pulmonary circulation times expressed as the number of heart cycles are shown in Table III. The mode was obtained for two-thirds to three-fourths of the animals in the various groups.

The deviations from the mode were not great. The number of heart beats needed to force the blood from the proximal part of the pulmonary artery to the left atrium increased with the age of the animals.

A comparison of the angiocardiographic films, electrocardiograms and pressure curves showed that extrasystoles, which are frequently seen in selective angiocardiography, had no, or only a slight, ability to transport the blood into the pulmonary vessels. On the other hand, determinations of the volume of the left ventricle revealed that the first beat after the extrasystole started from a greater end-diastolic volume and resulted in a greater stroke volume than the beats before the extrasystole.

Table III. Pulmonary circulation times in seconds and in numbers of heart cycles

Extrasystoles are indicated by *x*

Animal group	Sec.	Number of cycles	Range
2—3-month-old puppies	1.0	$2 + x$	$2 - (2 + x)$
Full-grown dogs	1.7	4	$4 - 5$

The occurrence of extrasystoles did not, however, lead to any uncertainty in the results of the experiments. In the puppies the pulmonary circulation time never exceeded two heart cycles and an extrasystole, but in the full-grown dogs the circulation time was 4—5 cycles.

Discussion

From the results presented it appears that the relative stroke volume (as a percentage of the end-diastolic volume) decreases, and the residual blood volume increases with age. The stroke volume is a most important indicator of the heart work and several investigators have studied the stroke volume in adults and children. With the introduction of cineangiocardiography it was possible for the first time to determine simultaneously the stroke volume and the amount of residual blood in the left ventricle. The method was used for studying the volume of the left ventricle by RUSHMER and THAL in 1951 and by CHAPMAN and BAKER in 1958. The former did not calculate the volume as such,. but used the ventricular area in a single plane as an index to the volume. The latter presented both the method and the results from two cases, dog and man. The results, however, were very different. The residual blood volume was determined to 15 per cent in the man and 65 per cent in the dog.

Determinations of stroke and residual blood volumes in full-grown dogs have previously been presented by GRIBBE et al, Good correlation between the Fick direct method for determining the minute volume was established.

Until now, no results have been published on comparative studies of the residual blood volume between fullgrown and young subjects.

The striking difference demonstrated between the relationship stroke volume/residual blood volume in growing and full-grown subjects is apparent.

The young heart cannot meet increased requirements except by increasing the heart rate (REIN and SCHNEIDER 1956).

The pulmonary circulation time has been discussed only in a few reports. In 1927 BLUMGART and WEISS employed Radium C to determine the pulmonary circulation time in man. They found the average pulmonary circulation time in adults to be 6.5 sec or 7—8 heart beats. In 1953 NERI et al. used fluorodensography for this purpose and observed that the circulation time from the right

ventricle to the left ventricle takes 4 sec or 31/2 heart beats on an average. In 1954 NORDENSTRÖM measured the circulation time from the pulmonary artery to the left atrium in full-grown dogs by densitometry and found it to vary from 2.1 to 3.5 sec. It can be calculated from his figures that this corresponds approximately to 6—9 heart beats. It is possible that the first signs of contrast medium are seen earlier in the left atrium in angiocardiographic films than they are observed by the above method, and hence this may be the reason why we found a smaller number of heart beats in our dog series. In connection with roentgencinematographic angiocardiography on man JANKER and HALLERBACH (1951) observed a pulmonary circulation time of 3.29 sec which corresponds to four heart cycles.

We were unable to find any information from the literature about comparative studies of the pulmonary circulation time in different age-groups. Our experiments showed a prolongation of the pulmonary circulation time with advancing age and body size, not only in time units but also when it was expressed as the number of cardiac cycles. This prolongation was probably associated with the change with age in the relative stroke volume. The mean stroke volume was 0.75 per mille of the body weight for the full-grown animals, which was 40 per cent lower than the mean stroke volume, 1.23 per mille, for the puppies.

Other factors *e. g.* pulmonary vascular resistance, pulmonary blood pressure, short circuits, blood volume in the pulmonary vessels and the relative size of the heart and lungs at various ages may exert an influence on the pulmonary circulation time. This study could not take into account the possible importance of these factors.

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Effects of Intravenous Infusions of Adrenaline and Noradrenaline on Certain Psychological and Physiological Functions

by

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Abstract

FRANKENHAEUSER, M., G. JÄRPE and G. MATELL. *Effects of intravenous infusions of adrenaline and noradrenaline on certain psychological and physiological functions.* Acta physiol. scand. 1961. *51.* 175—186. — Effects of intravenously infused l-adrenaline (average dose 14.9 μg/min, average infusion time 34.7 min) and l-noradrenaline (average dose 16.2 μg/min, average infusion time 36.8 min) were examined by objective methods in six subjects. The psychological tasks were chosen so as to represent different functional areas. Heart rate and arterial blood pressure were continuously recorded, and excretion of urinary catechols before, during and after infusions was determined. Both drugs produced subjective symptoms (mental and physiological), and these were more pronounced and more frequent for adrenaline. Marked changes in heart rate and arterial pressure were recorded during the infusions. The stress involved in performing the psychological tasks also caused a pronounced increase in heart rate, arterial pressure and the urinary excretion of catechols. Performance did not differ significantly from that during the infusion of Ringer's solution. These results were tentatively interpreted to indicate that an increased alertness accompanying the emotional changes produced by the drugs may act to counterbalance other, unfavourable effects on performance.

The main aim of the present experiments was to study effects of intravenously infused adrenaline and noradrenaline on certain psychological functions as measured by objective techniques.

Though the general effects of infused adrenaline on physiological processes and emotional state are well known, quantitative data as to effects on perform-

ance are scarce. JERSILD and THOMAS (1931) examined the effects of subcutaneous injections of adrenalin hydrochloride (0.4—0.9 mg) and found a slight improvement in motor tasks (tapping and strength of grip), but no change in other psychological functions. LANDIS (1935) investigated the influence of subcutaneous injections of adrenalin (1 mg adrenalin hydrochloride, Parke, Davis & Co) on complex muscular activity (dart throwing) expecting to find an impairment in speed and accuracy, but no change could be demonstrated. BASOWITZ et al. (1956) found that motor performance was significantly impaired during prolonged intravenous adrenaline infusion at a low dose level (5 μg/kg body wt/hr), whereas performance in other types of tasks was not affected.

Psychological effects of noradrenaline do not seem to have been studied by quantitative methods. However, the general opinion, based on observations and introspective accounts of subjects, appears to be that noradrenaline does not cause marked psychological changes (*e. g.* SWAN 1952).

Data provided by studies of performance in other types of stress-situations (failure stress, task-induced stress etc.) do not present a clear picture. The problem is highly complex and it is evident that stress may both aid and interfere with performance depending upon a number of factors, such as the degree and nature of the stress, the type of performance as well as various personality traits of the subject (LAZARUS, DEESE and OSLER 1952, STEINBERG 1959).

Some of the mental symptoms commonly occurring during infusion of adrenaline, such as increased mental alertness, may be expected to improve performance, whereas others, such as severe anxiety, may be accompanied by a deterioration in performance. Hence it did not seem possible to make predictions as to specific effects on performance. Neither could such predictions be made in respect of noradrenaline.

On the basis of these considerations the most adequate mode of approach seemed a relatively broad, explorative study which would provide more information as to the effects of these drugs on various aspects of behaviour. A battery of tests was put together, which included samples from several areas of psychological functioning: psychomotor performance, power of concentration, memory span, word fluency, time estimation and a colour-word test.

In addition, heart rate and arterial blood pressure were recorded and urinary excretion of adrenaline and noradrenaline was examined.

By examining each subject during infusion of adrenaline, noradrenaline and Ringer's solution (control condition) it should be possible to determine if certain aspects of behaviour are particularly sensitive to the drugs. Furthermore the concomitant physiological recordings should make it possible to relate physiological and psychological data to each other. Hence the information gained by these experiments should provide the necessary basis for further investigations on more specific problems.

Methods

Subjects

Six male university students participated in the experiments. Their ages ranged between 19 and 31 years (average 23.3 years), and body weights and heights between 63 and 71 kg (average 67.7 kg) and 177—190 cm (average 182.2 cm), respectively.

Infusions

Catecholamines and placebo solutions were given as a continuous i.v. drip during periods ranging between 33 and 37 min. The catecholamines were given in solutions containing 2 mg base per ml and were prepared immediately before each experiment from stock solutions of l-adrenaline HCl and of l-noradrenaline tartrate.[1] The total dose, as base, of infused adrenaline was 0.45—0.60 mg and the dose per minute averaged 14.9 μg. The corresponding figures for noradrenaline were 0.50—0.75 mg and 16.2 μg. The infusions were given in the left antecubital vein, into which a teflon catheter (Barr and Soila 1960) had been percutaneously introduced.

Physiological measurements

Heart rate. In all experiments heart rate was continuously recorded by means of an instantaneous cardiotachometer (Sturm and Wood 1947).

Arterial blood pressure. In the left radial artery another teflon catheter was percutaneously introduced (Barr and Soila 1960). The catheter was connected to a Statham strain gauge pressure transducer placed at heart level.

Both the heart rate and the arterial blood pressure were recorded photokymographically. An extra mirror galvanometer was connected to the strain gauge for visual monitoring of the arterial pressure. In addition the instantaneous heart rate could be read at any time on the cardiotachometer dial.

Urinary cathechols. Immediately after voiding, the urinary volume was measured and pH adjusted to 3.5 with N HCl. Specimens were then stored at + 4° C until analysis, which was performed using a fluorimetric technique (Euler and Lishajko 1959).

Psychological variables

The test battery comprised 7 tasks, which were always given in the order listed below.

100-7 test. The task was to count backwards from 100 by subtracting seven each time. The score was the time taken to complete the count.

Tapping speed. The task was to tap as rapidly as possible with the right hand index finger on a key connected to an electric counter. The score was the number of taps in 30 sec.

Time estimation. Present- and past-time estimates of 4 time periods between 9 and 37 sec were obtained using the method described by Frankenhaeuser (1959 p. 38 ff). Scores for present, past and retained time were calculated (cf. Frankenhaeuser 1959, p. 40).

Colour-word test. One part (card C) of the Stroop test (Thurstone and Mellinger 1953) was used. This consists of 100 colour words (blue, red, yellow, green) printed in random order on a gray sheet. The combination of words and colours is incongruent, so that the word yellow may be coloured red etc. The task is to name the *colour* of the print, ignoring the word, as quickly as possible. The score was the time taken to complete the task.

[1] Nor-Exadrin conc. ASTRA, Södertälje, Sweden.

Word fluency. The task was to write down as many words as possible that start with *s* and end with *a*. The time allowed was 3 minutes and the score was the number of words correct.

Mirror drawing. The task was to move a stylus along a track cut out in a brass plate so as to form a 5-pointed star. This track was visible only in a mirror. When the stylus touched the margins of the track a circuit was completed (for details see FRANKENHAEUSER, HESSER and GRAFF-LONNEVIG 1960). A time score was calculated as the mean of the time taken to complete 4 consecutive trials and an error score as the mean time of contact between stylus and margins.

Memory span. The task was to repeat digits read by the examiner a) forwards, and b) backwards. The scores were the maximum number of digits correctly repeated.

Peg-washer test. (DUREMAN and SÄLDE 1959, p. 17). The task was to move nobbed pegs from one side to another of a drilled board. A washer had to be fitted on each peg before it was replaced on the board. The time score was the time taken to move 50 pegs, and the error score the number of washers and pegs dropped.

Experimental design

Each subject came to three experimental sessions, in which infusions of adrenaline, noradrenaline and Ringer's solution, respectively, were given. The procedure was exactly the same each time and the subject did not know which infusion was given. Each subject had his three sessions at the same time of the day and the intervals between sessions ranged between 5 and 8 days.

In order to counterbalance effects of practice on performance and of general adjustment to the experimental situation, the three conditions were rotated as follows.

Subject	Session I	Session II	Session III
1	Adrenaline	Noradrenaline	Ringer's solution
2	Noradrenaline	Ringer's solution	Adrenaline
3	Ringer's solution	Adrenaline	Noradrenaline
etc			

This design was repeated for the three remaining subjects.

Under each condition the procedure was as follows. The subject emptied his bladder. Preparations were made for intravenous infusion and recording of blood pressure and heart rate. The first sample of urine was obtained after about 1 hour, when all preparations for the experiment were completed. The subject was comfortably seated and his ability to move his arms and hands freely and without pain was checked. He was told that the infusion would begin shortly and asked to report any symptoms that occurred. (Measuring units and infusion aggregate were placed behind the subject so as to prevent him from following the procedure.) Some minutes later the infusion was started without further announcement to the subject. A few minutes after the infusion had begun the psychological testing was started. It was completed in about 30 minutes upon which the infusion was immediately interrupted and a second sample of urine was collected; this sample represented an average span of 40 minutes. The subject was told to relax while the recordings of heart rate and blood pressure were continued for about 25 minutes, when a third sample of urine was collected. Finally the subject was questioned about his symptoms and experiences before, during and after the infusion. The questioning followed a special check-list of symptoms and was thus invariably the same for all subjects under all conditions.

Table I. Subjective symptoms during adrenaline and noradrenaline infusions

	Adrenaline infusion Subject							Noradrenaline infusion Subject							Ringer's infusion Subject						
	1	2	3	4	5	6	f	1	2	3	4	5	6	f	1	2	3	4	5	6	f
Mental symptoms																					
Restlessness .	+	+	+	+	+		5	+			+	+		3							0
Agitation ...	+	+			+		3					+		1							0
Anxiety	+						1							0							0
Tenseness ...	+	+		+	+		4	+			+	+		3							0
Discomfort ..	+	+		+			3	+			+			2							0
Physiological symptoms																					
Palpitation ..	+	+	+	+	+	+	6		+	+	+	+	+	5					+		1
Tremor	+	+	+	+	+	+	6							0							0
Perspiration .	+			+			2				+			1							0
Numbness ..	+			+	+		3				+			1							0
Dyspnoe	+			+	+		3				+	+		2							0
Pounding in head, abdomen and thorax	+	+		+			3		+		+			2							0

Results

Subjective symptoms

The subjective symptoms experienced during adrenaline infusions had the same general characteristics as those described in previous investigations on healthy human subjects (*e. g.* EULER, LUFT and SUNDIN 1954, DUREMAN and SCHOLANDER 1956). Noradrenaline produced some effects similar to those of adrenaline, but these symptoms were on the whole less pronounced and less frequent. Symptoms mentioned by the subjects, either spontaneously or in response to the check-list questions, are listed in Table I. The table shows that there are large differences between subjects in frequency of symptoms. Subject 1, for example, reported 5 mental and 6 physiological symptoms, whereas subject 6 had no mental and only 2 physiological symptoms. The only symptom reported during infusions of Ringer's solution was one case of palpitation.

Effects of infusions on psychological variables

Though some subjects felt strongly affected by adrenaline, all were able to cooperate satisfactorily in the psychological testing. The mean scores are summarized in Table II. For those tests in which scores are obtained in physical time, and where consequently the unit of measurement is constant, performance

Table II. Means and S.E. of scores in performance tests during infusions of adrenaline, noradrenaline and Ringer's solution. Figures indicate time in sec except for Tapping, Word-fluency, Memory span and Peg-washer, error, where frequency is indicated

Variable	Scores during infusion of		
	Adrenaline	Noradrenaline	Ringer's solution
100—7 test	15.8 ± 2.37	21.5 ± 6.15	20.0 ± 3.97
Tapping speed	221.5 ± 8.71	228.4 ± 11.4	223.8 ± 5.97
Colour-word test	79.0 ± 6.42	80.7 ± 7.86	86.2 ± 9.65
Word-fluency	28.0 ± 3.47	31.0 ± 3.20	31.8 ± 4.41
Mirror drawing, time	15.9 ± 1.62	14.7 ± 1.02	14.3 ± 1.36
Mirror drawing, error	3.9 ± 0.33	3.3 ± 0.45	3.1 ± 0.08
Memory span:			
digits forwards	7.8 ± 0.32	7.5 ± 0.50	7.5 ± 0.43
digits backwards	6.2 ± 0.70	6.0 ± 0.58	6.2 ± 0.47
Peg-washer test, time	184.4 ± 18.40	175.3 ± 10.90	170.3 ± 15.10
Peg-washer test, error	2.4 ± 0.96	1.2 ± 0.40	1.2 ± 0.31

under adrenaline and noradrenaline conditions has also been expressed as per cent of performance during Ringer's infusion (Table III). The most striking result is that the differences in performance under the various conditions were on the whole small, in many cases even negligible. None of the differences reached statistical significance.

If the results are taken at their face value, they seem to indicate that the effects of adrenaline are stronger than those of noradrenaline and that adrenaline may improve performance in tests where speed plays a decisive role, except in the case of psychomotor ability, where performance seems impaired. However, the differences obtained may be due to chance and a much larger material would be required to confirm these tentative interpretations.

In time estimation a small but consistent difference between adrenaline and control scores was obtained, present- and past-time estimates both being

Table III. Performance (time scores) during infusions of adrenaline and noradrenaline in per cent of performance during infusion of Ringer's solution

Variable	Adrenaline infusion	Noradrenaline infusion
100—7 test	79	108
Colour-word test	92	92
Mirror drawing, time	111	103
Mirror drawing, error	126	106
Peg-washer test, time	108	103

Table IV. Means and S. E. of heart rate, systolic and diastolic blood pressure before, during and after infusions of adrenaline and noradrenaline

Variable	Adrenaline infusion			Noradrenaline infusion		
	Before	During	After	Before	During	After
Heart rate	78 ± 6	99 ± 8	91 ± 6	77 ± 5	63 ± 4	76 ± 8
Systolic blood pressure....	145 ± 5	190 ± 7	136 ± 7	126 ± 4	166 ± 8	122 ± 6
Diastolic blood pressure ..	83 ± 3	88 ± 5	80 ± 7	77 ± 4	96 ± 7	73 ± 3

larger during adrenaline. This effect is analogous to that produced by metamphetamine (FRANKENHAEUSER 1959). The influence of adrenaline on time estimation will be examined further in a separate series of experiments.

Effects of infusions on physiological variables

The mean heart rate, systolic and diastolic blood pressure of each subject before, during and after each infusion was calculated. The means of the individual means thus obtained are shown in Table IV. The changes seen are in general agreement with those obtained by previous investigators (*e. g.* EULER et al. 1954, GOLDENBERG et al. 1948). The only unusual feature is the rise (instead of expected fall) in diastolic pressure under adrenaline. A possible explanation is that the rise was caused by the combined effects of the infused adrenaline and the stress produced by the psychological tests (cf. p. 8).

The results of the analyses of urinary catechols are shown in Table V. It should be noted that the adrenaline values *before* infusions are higher than those obtained by previous investigators (EULER and LUFT 1951, EULER et al. 1954). This may be a consequence of the stress induced by the catheterization of blood vessels and it may also be an effect of anticipation of the experiment of follow.

Table V. Means and S. E. of catecholamine excretion in urine before, during and after infusions of adrenaline and noradrenaline

Variable	Adrenaline infusion			Noradrenaline infusion		
	Before	During	After	Before	During	After
Excreted adrenaline mμg/min	13.3±2.7	598.2±30.1	236.8±32.6	20.2±4.3	52.0± 6.3	29.5± 8.5
Excreted noradrenaline mμg/min	21.6±5.4	0.9± 1.0	14.9± 3.9	28.4±4.3	478.4±68.2	92.6±23.9

Table VI. Means and S. E. of heart rate, diastolic and systolic blood pressure and the excretion of catecholamines in urine before, during and after infusions of Ringer's solution with and without concomitant psychological testing

Variable	Infusion of Ringer's sol. plus psychol. testing			Infusion of Ringer's sol. only		
	Before	During	After	Before	During	After
Heart Rate.....	73 ±5	78 ±6	70 ±5	72 ±4	68 ±4	69 ±3
Systolic blood pressure.....	124 ±7	132 ±9	125 ±8	118 ±4	124 ±4	119 ±4
Diastolic blood pressure	66 ±4	73 ±4	67 ±4	81 ±2	83 ±4	85 ±2
Excreted adr. in mμg/min..	16.6±3.1	35.4±7.0	13.5±4.5	14.0±2.4	15.3±2.7	12.2±2.6
Excreted noradr. in mμg/min	21.2±4.1	31.2±3.7	20.3±5.3	18.7±2.5	16.2±0.8	15.2±2.5

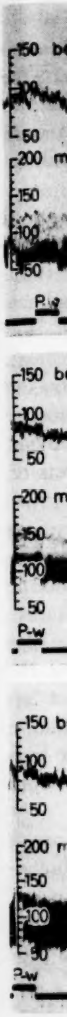
A second striking feature is the large amount of adrenaline excreted during the infusion period. The per cent output of infused adrenaline averages 5.01 (range 4.17—5.61), which is considerably more than that found in earlier investigations (EULER et al. 1954). In respect of noradrenaline the corresponding values are 3.22 % (range 2.38—4.17) which is consistent with earlier findings (EULER and LUFT 1951).

Effects of psychological tests on physiological variables

During the infusions of Ringer's solution an increase in heart rate, blood pressure and excreted catechols was noted. In order to find out if these changes were caused by the infusion *per se*, or by the psychological tests, or both, a new series of experiments with infusion of Ringer's solution only was carried out with the same six subjects. The procedure was exactly the same as before except that the psychological tests were substituted by the reading of various magazines with a neutral and unengaging content. Table VI shows the results from both series with Ringer's solution.

In the second series (without testing) the differences between measures obtained before and during infusion are either much less pronounced or abolished. Hence it may be concluded that the changes in the first series were primarily caused by the psychological tests and not by the infusion.

Another striking example of physiological reactions to the stress induced by psychological tests was seen in the continuous recordings of arterial pressure and heart rate as illustrated in Fig. 1. Performance — particularly in stressful tasks, such as the Colour-word test and Mirror drawing — is accompanied by distinct rises in systolic blood pressure. The figure also shows the rise in blood pressure caused by a test (Peg-washer) given before and after the infusions.



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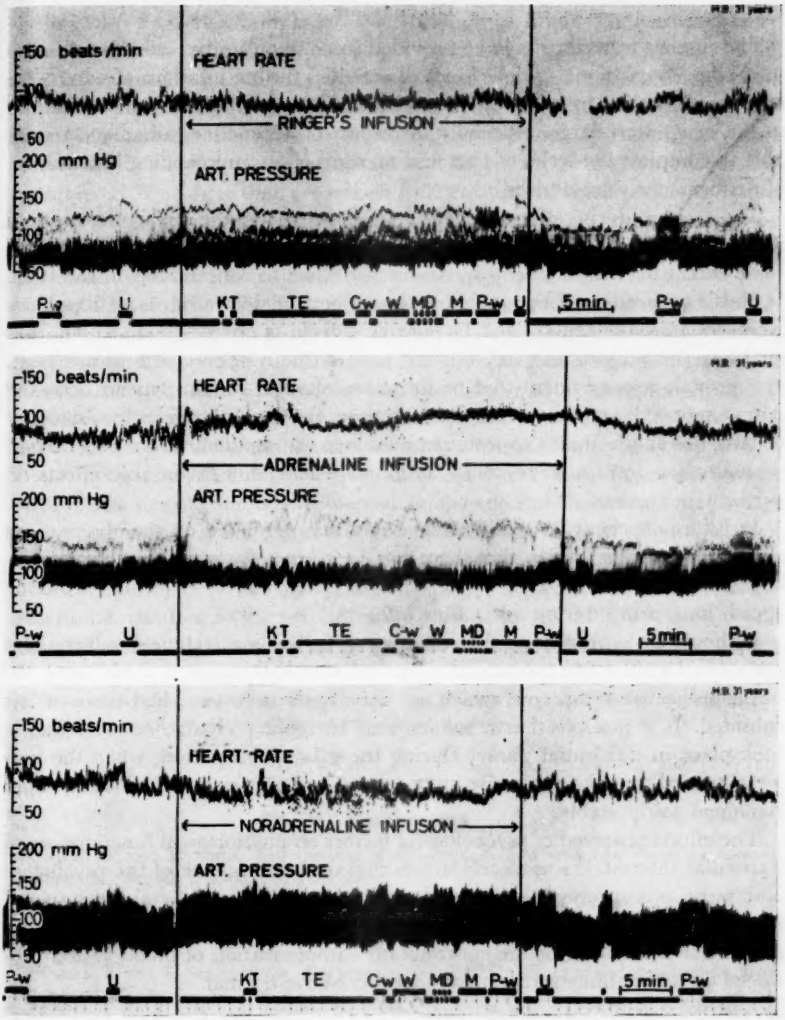

Fig. 1. Recordings of heart rate and arterial blood pressure before, during and after infusions of adrenaline, noradrenaline and Ringer's solution. Time for psychological tests is marked in the bottom tracing. P-w = Peg-washer test; U = Urine sample (the rise in blood pressure is in this case caused by the subject's standing up); K = 100-7; T = Tapping speed; TE = Time estimation; C-w = Colour-word test; W = Word fluency; MD = Mirror drawing; M = Memory span.

Discussion

The present experiments have provided some quantitative information as to the ability to carry out various kinds of activities during infusions of adrenaline and noradrenaline in relatively large doses. Though inter-individual reactions to the drugs were large, especially in respect of adrenaline, all subjects were able to complete the series of tests and no signs of an approaching break-down of performance were noticed.

The tremor during the adrenaline infusions made the psychomotor tasks more difficult to perform. However, most subjects seemed to compensate to some extent for this handicap by an intense effort to concentrate on the tasks. It thus appeared as if the unfavourable effects of decreased hand steadiness were counterbalanced by the favourable effects of increased alertness, thus enabling the subjects to carry out the tasks without appreciable impairment. However, it seems evident that performance changes should depend upon the doses employed, and that with increasing doses the negative effects would sooner or later dominate. It also appears reasonable to assume that, on the other hand, with smaller doses a stage could be found where only favourable effects on performance ensue.

In future experiments specific hypotheses may be based on the observations made in the present series, that there was a tendency for psychomotor performance to deteriorate and for other types of performance, requiring primarily speed, to improve during adrenaline infusion.

It should be kept in mind that the testing (for various technical reasons) was not started until a few minutes after the infusion had begun, *i. e.* not until the initial subjective symptoms, which are usually the strongest, had more or less subsided. It is possible that a sudden and temporary change in performance took place in this initial phase. During the subsequent period, when the tests were carried out, the subjective state as well as heart rate and blood pressure remained fairly stable.

The effects observed of psychological factors on physiological functions are of particular interest. It was clearly shown that the performance of the psychological tasks was accompanied by an increase in heart rate, arterial pressure and urinary excretion of catechols. Furthermore the stress involved in anticipation of the test period and/or the concomitant catheterization of blood vessels produced an adrenaline excretion considerably above normal.

A striking feature of the adrenaline experiments was the high percentage adrenaline recovered in the urine. One possible explanation may be that the diuresis was large in most cases because of extra water intake to secure prompt micturition. This interpretation is supported by a statistically significant positive correlation between diuresis and amount of catechols in the urine.

There has been considerable debate as to the question whether the emotional experiences produced by adrenaline injections are "real" emotions or whether

they are more adequately described as "cold" or "as-if" emotions, *i. e.* the subject feeling *as if* he were afraid, anxious etc. though he is not. The various observations have been critically reviewed by LANDIS and HUNT (1932), who conclude that injections of adrenaline but rarely produce genuine emotions. In the present experiments the emotional reactions to adrenaline were definitely of the "as-if" type. Furthermore the subjects appeared very reluctant to admit any severe emotional changes and seemed to attempt to ignore their symptoms as far as possible. It is evident that a successful penetration of these problems requires more refined techniques and at present various methods (ratings, interviews, projective techniques) for measurements of emotional experiences during adrenaline and noradrenaline infusions are being tried out.

Possible selective effects of adrenaline versus noradrenaline infusions on emotional changes are of particular interest in view of the relationships observed between certain specific psychological and physiological response patterns, fear being associated with "epinephrine-like" and anger with "norepinephrine-like" cardiovascular reactions (cf FUNKENSTEIN 1956). More direct evidence for a selective function of adrenaline and noradrenaline in emotion has been provided by studies where excretion of urinary catechols during stress has been compared with the nature of emotional responses. Such experiments indicate that anxiety, versus aggression, is related to a relatively larger increase in adrenaline versus noradrenaline. (ELMADJIAN, HOPE and LAMSON 1957, SILVERMAN, COHEN and ZUIDEMA 1957). In the present experiment qualitative differences in emotional reactions to the two drugs were not observed. It is, however, possible that differences may be revealed by special techniques, such as suggested above.

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Acta physiol. scand. 1961. 51. 187—192

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On the Nervous Regulation of the Biliary System in the Cat

By

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Abstract

PALLIN B. and S. SKOGLUND, *On the nervous regulation of the biliary system in the cat.* Acta physiol. scand. 1961. *51.* 187—192. — The nervous regulation of the biliary system was investigated on cats. Stimulation of the vagal nerves was shown to increase the pressure in the gallbladder and the hepatic duct, while stimulation of the splanchnic nerves was found to inhibit the action of the vagal nerves. From these experiments is concluded that the vagal nerves exert a 'secretory' action on the biliary system, while the splanchnic nerves inhibit the outflow of bile. The mechanisms underlying the interaction of these facilatatory and inhibitory actions are briefly discussed.

It is well known that the biliary system recieves its nerve supply from the right and left vagus nerves and the coeliac ganglia. Whether the phrenic nerves contribute any fibres to this innervation is not known. However, it has been shown by ALEXANDER (1940) that these latter nerves join the sympathetic and parasympathetic rami which enter the liver. The nerves directed to the biliary system accompany the hepatic artery and the portal vein and according to ALEXANDER (1940) the afferent innervation of the blood vessels is sympathetic and the innervation of the bile ducts both sympathetic and parasympathetic. The function of these nerves have been the subject of some investigations (TANTURI and IVY 1938, JOHNSON and BOYDEN 1943, POILLEUX *et al.* 1952). In these works it was shown that the vagus nerves are 'secretory', that is, when stimulated bile is released from the biliary system. If the bile-formation in the liver is under direct nervous control is uncertain, but TANTURI and

Table I.

Cat	IP	Right vagus stim.	Left vagus stim.
1	0	0.8	0.1
2	0	1.3	0.2
3	0	0.7	0.4
4	0	0.7	0.1
5	0	0.4	0.3
6 M	0	1.1	0.0
7 M	0	1.4	0.3
average		=0.9	=0.2

Table I showing the effect of vagal stimulation. The splanchnic outflow intact. Initial pressure (IP) set at zero. Pressure variations measured by a strain gauge manometer with a polyethylene catheter in the gallbladder. Figures demonstrate pressure alterations in cm H_2O.

Ivy (1938) suggested that there might be some direct nervous influence on the secretion of bile from the liver on dogs. Stimulation of the sympathetic nerves of the biliary system has been shown to exert an inhibitory action on the output of bile (Johnson and Boyden 1943) and to inhibit the output of bile from the liver (Tanturi and Ivy 1938).

The literature gives no further information about the nervous regulation of the biliary system and furthermore most of the knowledge has been gained from inspection of the volume variations of the gallbladder which must be considered a rough method. It was therefore considered worth-while reinvestigating the control exerted by different nerves acting on the biliary system; recording the effects as pressure variations of closed systems.

Methods

Fifty-two cats were used. They were anesthetized with Nembutal (Abbot) 40 mg/kg bodyweight intraperitoneally. A tracheal cannula was routinely inserted. Thoracotomy was performed on the right side. The tracheal cannula was connected with a pump for artificial respiration. The sixth to the tenth rib was resected to the sternal bone and the inferior lobe of the right lung was extirpated. The vagus nerves were dissected free just above the diaphragm. The abdomen was opened in the midline. This approach was used in all experiments.

In one series of experiments a polyethylene catheter was inserted into the gallbladder according to the technique demonstrated by Ivy and Janecek (1959) and connected to a sensitive inductive strain-gauge manometer. In some experiments indicated by "M" a manometer was used for pressure measurements according to the method by Ivy and Janecek (1959). In all the experiments the initial pressure (I. P., in the tables set at zero) was decided three times with an interval of three minutes. The cystic duct was dissected

Table II.

A.					B.		
Cat	IP	Spl. n. intact	Spl. n. cut	Diff.	Spl. n. intact	Spl. n. cut	Diff.
1	0	0.8	1.7	0.9	0.1	0.3	0.2
2	0	1.3	1.5	0.2	0.2	0.6	0.4
3	0	0.7	1.7	1.0	0.4	0.7	0.3
4	0	0.7	1.6	0.9	0.1	0.4	0.3
5	0	0.4	1.5	1.1	0.3	0.4	0.1
6 M	0	1.1	2.2	1.1	0.0	0.6	0.6
7 M	0	1.4	1.8	0.4	0.3	0.6	0.3
average				=0.8			=0.3

Table II showing effect of vagal stimulation (A, right vagus and B, left vagus) before and after sectioning of the greater, lesser and least splanchnic nerves. Initial pressure set at zero. Pressure variations measured by a straingauge manometer with a polyethylene catheter in the gallbladder. Figures demonstrate pressure alteration in cm H_2O.

free from vessels and ligated. The dissection was made very carefully not to exert pressure on to the gallbladder during the operation which causes bile outflow. The thoracic cavity was filled with paraffin oil at 37° C and kept at that temperature throughout the experiment.

In another series of experiments the same operative approach as in the first series was used but the polyethylene catheter was now inserted into the hepatic duct, which was dissected free from the vessels and the nerves. The pressure was recorded in the same way as described above. When using the IVY and JANECEK technique, the troachar was connected to a polyethylene catheter which was inserted into the hepatic duct.

In both series of experiments the dissected nerves were stimulated electrically by supramaximal shocks at frequencies between 5 and 36/sec. By recording the action potentials it was always checked that the nerves were supramaximally stimulated.

Results

In the first series of experiments, in which the pressure was measured in the gallbladder after ligation of the cystic duct, stimulation of the peripheral ends of vagal nerves in 7 animals showed, that the right one had a more potent effect than the left (see Table I). When the right vagus was stimulated, the pressure of the gallbladder increased 0.9 cm H_2O. When the left one was stimulated the rise was only 0.2 cm H_2O.

When these effects had been established the greater, lesser and least splanchnic nerves were cut before they reached the coeliac ganglia (7 animals). When now stimulating the right and left vagus the pressure in the gallbladder increased 0.8 cm and 0.3 cm H_2O respectively (see Table II). From this it can be concluded that the sympathetic fibres have some inhibitory action on the release of bile from the gallbladder.

Table III.

A.					B.		
Cat	IP	Symp. n. intact	Symp. n. cut	Diff.	Symp. n. intact	Symp. n. cut	Diff.
1	0	0.5	2.1	1.6	0.0	0.3	0.3
2	0	0.8	2.2	1.4	0.2	0.6	0.4
3	0	0.7	1.7	1.0	0.1	0.7	0.6
4	0	0.9	1.9	1.0	0.3	0.9	0.6
5	0	1.0	2.6	1.6	0.1	0.6	0.5
average				=1.3			=0.5

Table III showing the effect of vagal stimulation (A, right vagus and B, left vagus) before and after sectioning all branches contributing to the splanchnic nerves from th 4 to Ll and removing the sympathetic trunks from the diaphragm to the ganglion impar on both sides. Initial pressure set at zero. Pressure variations measured by a strain-gauge manometer with a polyethylene catheter in the gallbladder. Figures demonstrate pressure changes in cm H_2O.

In another series of experiments all branches contributing to the splanchnic nerves from Th 4 to L 1 were cut in 5 animals, and the whole sympathetic trunks from the diaphragm to the ganglion impar was removed on both sides. This operation was performed after having decided the effects of vagal stimulation in the intact animal. It was now found (see Table III) that the differences in effect on the pressure produced by vagal stimulation in the gallbladder was very small compared with those in which the splanchnic nerves had been cut. Consequently, the influence of direct sympathetic fibres on the gallbladder must be small.

In the experiments reported hitherto the effects of the sympathetic outflow from both sides have been considered. To determine any possible differences between the right and left side the splanchnic nerves on the right side were cut in 5 animals after having decided the effect of vagal stimulation in the intact animal. It was then found (see Table IV) that most of the inhibition exerted by the splanchnic nerves emanated from the right nerves.

In 6 animals only the greater splanchnic nerve was cut and the right and left vagal nerves stimulated peripherally. The pressure-rise now obtained was nearly as great as when all splanchnic nerves had been cut. From these experiments the conclusion can be drawn that the inhibition of the gallbladder from the lesser and least splanchnic nerves is less potent than that from the greater one.

In 4 animals the right vagus nerve was stimulated simultaneously with the right greater splanchnic nerve. The pressure in the gallbladder was then found to increase very much less than on vagal stimulation alone. This shows that the sympathetic influence on the gallbladder is inhibitory on the action of the parasympathetic.

Table IV.

A.					B.		
Cat	IP	Spl. dx. intact	Spl. dx. cut	Diff.	Spl. dx. intact	Spl. dx. cut	Diff.
1	0	0.9	1.5	0.8	0.1	0.3	0.2
2	0	0.9	1.7	0.8	0.3	0.7	0.4
3	0	0.6	1.3	0.7	0.0	0.3	0.3
4	0	0.9	1.4	0.5	0.3	0.4	0.1
5	0	0.7	1.6	0.9	0.5	0.6	0.1
average				=0.7			=0.2

Table IV showing the effect of vagal stimulation (A, right vagus and B, left vagus) before and after sectioning of the splanchnic nerves on the right side. Initial pressure set at zero. Pressure variations measured by a straingauge manometer with a polyethylene catheter in the gallbladder. Figures demonstrate pressure changes in cm H_2O.

TANTURI and IVY (1938) described a reflex in which stimulation of the central end of one vagus nerve with the other intact resulted in an increase of the flow from the liver. They could not produce this reflex in the cat. This experiment was here repeated while measuring the pressure in the gallbladder. In 4 animals the left vagus was cut and stimulated centrally. A pressure-rise of 0.3 cm H_2O was recorded in the gallbladder. In another series of 4 animals where a pressure-rise was first established in the intact animal, the right vagus nerve was cut and now stimulation at the central end of the left vagus nerve caused no pressure-increase in the gallbladder.

In another type of experiments the catheter was inserted into the hepatic duct and the vagal nerves were stimulated before and after section of the sympathetic outflow to the liver. The aim of these experiments was to study the effects that could be induced on the release of bile.

The vagal nerves were stimulated in 7 animals as described in earlier experiments. A pressure-increase of 0.7 cm and 0.2 cm H_2O respectively could be produced, which shows that the parasympathetic nerves in some way increase the intrahepatic pressure. The rise was obtained after stimulating a few minutes.

In 7 animals all splanchnic roots were cut before they reach the coeliac ganglia and when now stimulating the right and left vagus nerves peripherally the pressure-increase recorded in the hepatic duct was greater than before nerve-section. The splanchnic nerves therefore must have an inhibitory action on the effects that the vagal nerves exert on the liver.

In another 7 animals all splanchnic nerves were cut, the sympathetic trunks removed, and the phrenic nerves cut in addition. Stimulation of the right and left vagus nerves at their peripheral ends now resulted in a greater pressure-rise than with section of the splanchnic nerves alone. The phrenic nerves and the sympathetic trunks thus seem to have some inhibitory action on the pressure-increasing effect of the vagal nerves.

Discussion

The results presented show that the vagal nerves act as 'secretory' nerves, that is when stimulated they increase the pressure in the gallbladder. This indicates that these nerves have a tonic effect on the wall of the gallbladder. On the other hand the splanchnic nerves exert an inhibitory action on the tonus and decrease the effect induced by the vagal nerves.

The pressure-variations obtained on stimulation of the vagal nerves were small, but this was not unexpected because such small variations were also found by IVY and JANECEK (1959) when they stimulated the gallbladder-emptying mechanism by the use of cholecystokinin.

The results presented partly confirm earlier observations by TANTURI and IVY (1938) and JOHNSON and BOYDEN (1943). JOHNSON and BOYDEN did only determine the time necessary for gallbladder-evacuation by X-ray technique. They found by nerve-sections that the vagal nerves must have a 'secretory' action which is inhibited by the splanchnic nerves. In their experiments the sphincter of Oddi was included. In our experiments, on the other hand, the cystic duct was ligated thus leaving out any effects on the sphincter of Oddi. The ligation of the cystic duct might destroy some nerve-fibres running to the gallbladder, but as the major part accompanies the cystic artery, which was dissected free, most of the innervation was probably intact.

TANTURI and IVY (1938) demonstrated a reflex-action via the vagus on the release of bile from the liver in the dog. This was not investigated here, but a reflex acting via the vagus on the gallbladder could be demonstrated.

In the experiments where the pressure was recorded it was found that stimulation of the vagal nerves increase the release of bile from the liver. TANTURI and IVY (1938) reported a direct effect of vagal impulses on bile-production on dogs. However, they could not produce this effect in the cat. These effects, might, however, as suggested by TANTURI and IVY and MALLET-GUY *et al.* (1952) be induced by blood pressure or blood flow variations in the liver. The possible mechanisms behind these effects will be taken up in further work like the interesting facilitating and inhibiting mechanisms acting on the gallbladder-tone, which seem to present an excellent experimental situation for the testing of humoral and nervous intergration.

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Acta physiol. scand. 1961. 51. 193—203

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Noradrenaline Release from Isolated Nerve Granules

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Abstract

EULER, U. S. v. and F. LISHAJKO. *Noradrenaline release from isolated nerve granules.* Acta physiol. scand. 1961. *51.* 193—203. — Some properties of isolated transmitter granules obtained by high *g* centrifugation of press juice from bovine splenic nerves have been studied. Of the total noradrenaline present in the press juice 20—35 per cent was recovered in the sediment. The granules are stable for several hours at + 4° C but rapidly lose their noradrenaline on incubation in isotonic neutral media at 37° C. Noradrenaline is rapidly released even at low temperature at pH 4 and below, and by detergents. Hypo- and hyperosmotic solutions and freezing and thawing had a moderate releasing effect. Acetylcholine, nicotine, serotonin and GABA had no detectable effect on the noradrenaline release from the granules. Nerve granules are more sensitive to temperature than adrenal medullary granules but show higher resistance to freezing and thawing and osmotic changes.

High speed centrifugation of homogenates of bovine splenic nerves yields a sediment which contains noradrenaline in high concentration (EULER and HILLARP 1956). This finding has been confirmed by SCHÜMANN (1958), who also demonstrated the presence of ATP in the sediment, suggesting similar properties of the nerve granules as in those from adrenal medulla (FALCK, HILLARP and HÖGBERG 1956).

EULER and HILLARP (1956) further showed that the transmitter granules isolated from adrenergic nerves are relatively stable in isotonic solutions but readily give up their contents when exposed to acids.

The specificity of the granules was indicated by the fact that they did not contain histamine, which was present in large quantities in the original material. Chromatographic analysis of extracts of the separated granules gave no indication of the presence of other catechol compounds (EULER 1958), nor did biological tests reveal actions of any other kind than noradrenaline.

Previous experiments (EULER 1958) indicated that heating to 100° C, addition of acids and detergents cause a release of the noradrenaline contained in the particles.

In the present paper some results obtained with adrenergic nerve granules prepared by an improved technique will be described.

Methods

Bovine splenic nerves obtained at the slaughter house were dissected free from adjacent tissue and the nerve sheath removed. Preparations were made within 2 hours after the death of the animal. The nerves, usually 3—10 g, were collected on ice, wrapped in gauze cloth and squeezed between nylon cylinders. The press juice together with the washing fluid (see below), about 10 ml per g of nerve, was collected on ice and centrifuged for 5 min at about 1,000 × *g* and the sediment discarded.

As washing fluid for the primary suspension, sucrose in 0.3 M solution was used initially but later abandoned since it was observed that the yield was similar when sodium chloride or potassium phosphate were used as suspension media. Sedimentation in sucrose required higher *g* and longer centrifugation time; moreover, sucrose interfered with the fluorimetric assay of noradrenaline.

Plasma has also been tried as suspension medium but did not seem to offer any advantages.

In the majority of the later experiments 0.075—0.13 M potassium phosphate of pH 7.2—7.3 was used as washing fluid for the primary suspension. This was then centrifuged for 30 min at about + 5° C at 50,000 × *g*. The supernatant was adsorbed on alumina and eluted with acetic acid for fluorimetric estimation of noradrenaline according to EULER and LISHAJKO (1959).

After careful drying of the walls of the centrifuge tubes with moistened filterpaper, the sediment was resuspended in various media. These were allowed to act for certain time periods and at different temperatures as described in the text. Thereafter the suspension was again centrifuged for 30 min in which time complete resedimentation was achieved. The supernatant was used for fluorimetric assay of noradrenaline.

Extraction of sediment

The noradrenaline present in the sediment could be quantitatively extracted with 0.1 N HCl added to give pH 2. By addition of 0.2 ml 2.5 % HPO_3 the granules could be sedimented by centrifugation at 10,000 g for 5 min. With HCl alone or with perchloric acid, sedimentation required longer time at high *g*. A lower yield was regularly observed when extraction was made with HCl at pH 0.5—0.7 than at pH 2. Trichloroacetic acid was unsuitable for extraction of the granules since it interfered with the fluorimetric assay. When acid alcohol was used for extraction it was found that some activity remained in the precipitate.

The noradrenaline present in the extract of the granules was estimated by the fluorimetric method of EULER and LISHAJKO (1959).

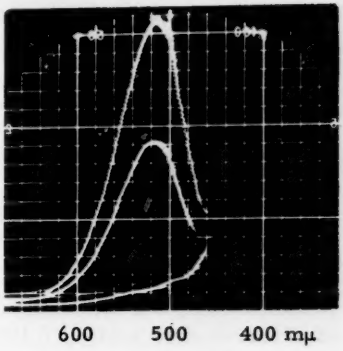

Fig. 1. Fluorescence spectra of extract of sediment from press juice from bovine splenic nerve (upper curve) noradrenaline (middle curve) and blank (lower curve) after transformation to lutines. Activating wavelength 395 mμ.

Results

Catechol compounds in supernatant and sediment from press juice of bovine splenic nerves

In a previous paper (EULER 1958) it was reported that chromatography of an extract from sediment obtained by high *g* centrifugation of press juice from bovine splenic nerves only revealed the presence of noradrenaline. If the catechol compounds present in extracts of the sediment were condensed with ethylene diamine or oxidized to lutines and subjected to spectrophotofluorimetric analysis (Aminco) evidence was obtained for the presence of noradrenaline only (Fig. 1).

In 4 experiments the supernatant from centrifugation of suspensions of bovine splenic nerve granules in 0.3 M glucose was passed through an alumina column and the catecholamines eluted with 0.25 N sulphuric acid. The con-

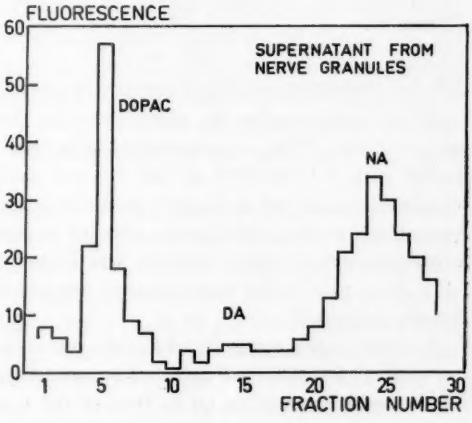

Fig. 2. Starch column chromatogram of supernatant after high *g* centrifugation of microgranule suspension from bovine splenic nerves. Solvent: n-butanol-acetic acid-n/10 HCl (5 : 1 : 1). Ordinate, fluorescence in arbitrary units after condensation with ethylene diamine. Abscissa, fraction number. DOPAC = 3 : 4, dihydroxyphenylacetic acid, DA = dopamine, NA = noradrenaline.

Table I. Total noradrenaline (NA) obtained from bovine splenic nerves by squeezing, percentage NA in the sediment and release during incubation for 30 min at + 20°C. Mean and S. D. from 15 exp.

Total amount of NA per g nerve obtained by squeezing	Mean recovery of NA in sediment in per cent of total	Mean release of NA on incubation 30 min + 20°C in per cent (controls)
8.7 ± 1.35 μg/g	28.3 % ± 4.5 %	33 % ± 7.6 %

centrated eluate was chromatographed on a starch column in a solvent system of n-butanol-acetic acid -n/10 HCl (5 : 1 : 1). Fig. 2 shows the relative fluorescence of aliquots of the different fractions after condensation with ethylene diamine. The fluorescence peaks agreed well with those of dihydroxyphenyl acetic acid (fraction 4—6) and with noradrenaline (fraction 22—30). It was particularly noteworthy that the dopamine fluorescence peak was well marked only in 2 of the experiments, suggesting that there is no direct quantitative relationship between dopamine and the nerve transmitter.

Total yield of noradrenaline, recovery in sediment, and release on incubation

The total amount of noradrenaline obtained from bovine splenic nerves by the squeezing technique was on an average 8.7 μg per g which compares well with the amount obtained by extraction with trichloroacetic acid and fluorimetric estimation (uncorrected). The results thus indicate that the yield was nearly maximal. In 15 consecutive experiments, in which the sediment after resuspension in 0.075 M potassium phosphate was incubated for 30 min at + 20° C, the percentage total recovery of noradrenaline in the sediment and the proportion released during incubation in the controls was estimated (Table I).

Effect of temperature and time of exposure on granules

At low temperature the granules retain their contents of noradrenaline for several hours. When a suspension made from bovine splenic nerves was prepared with 0.15 M KCl at pH 7.0 and centrifuged immediately the amount of noradrenaline per g of nerve in the sediment was 2.1 μg in two samples. In two other samples which were allowed to stand for 180 min at + 4° C after centrifugation the same amount was found. Even after 5 hours exposure at + 5° C at pH 7.7 the noradrenaline amount in the sediment was only insignificantly decreased.

In other experiments in which the suspension at pH 6.8 — 7.7 was exposed to + 4° C for 180—300 min before centrifugation, the noradrenaline content of the sediment was equal to that of the controls (centrifuged immediately)

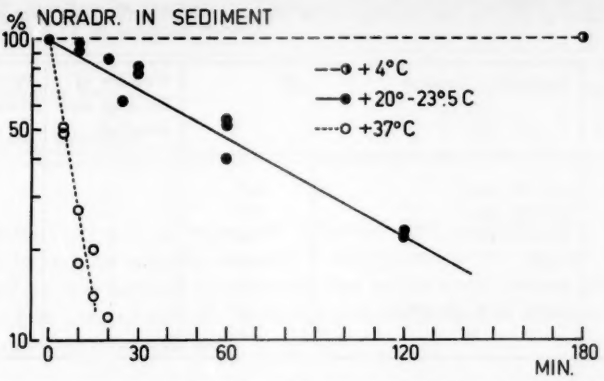

Fig. 3. Effect of temperature on noradrenaline release from isolated granules from bovine splenic nerves incubated in 0.13 M potassium phosphate or 0.15 M Na Cl at pH 6.8—7.7.

(Fig. 3). The release on incubation at + 20° C proceeded at a faster rate so that 70—80 per cent of the activity was liberated after 2 hours exposure. No difference was found with 0.15 M NaCl or 0.13 M potassium phosphate solution as incubation media. At 37° C the release proceeded at a much faster rate as seen in the figure.

Freezing and thawing

It was shown by HILLARP and NILSON (1954) that when chromaffin cell granules from the adrenal medulla were exposed to freezing and subsequent thawing a considerable portion of their amine content was released.

In a series of experiments the effect of freezing and thawing was studied on splenic nerve granules either in suspension or in the sediment after centrifugation. The samples were frozen by placing the original suspension or the resuspended sediment in a deep-freeze at — 26° C, followed by thawing at room temperature. In some experiments the freezing and thawing was repeated up to 6 times. Controls were kept at + 3—5° C where the spontaneous loss was negligible. In all instances the releasing effect of this treatment was only moderate, as illustrated in Table II.

As seen in the table the extra release caused by freezing and thawing reached 50 per cent only in one case in which the process was repeated 5 times with distilled water as suspension medium. In 0.15 M KCl repeated freezing and thawing had no more effect than a single treatment, and in 0.075 M potassium phosphate the loss from the sediment was 21 and 33 per cent compared with the controls. In those instances (exp. 4 and 5) where noradrenaline was also determined in the supernatant after recentrifugation the deficit observed in the sediment after freezing and thawing was found in the supernatant.

Table II. Effect of freezing and thawing on the release of noradrenaline from nerve granule suspensions

Exp. no.	Suspension medium	pH	Number of freezings and thawings	Percentage release
1	0.15 M KCl	6.7	1	25
2a	0.15 M KCl	6.7	6	28
2b	0.15 M KCl	7.0	1	19
3a	H_2O	6.8	1	28
3b	H_2O	6.8	5	50
4	0.075 M K-phosphate	7.6	1	33
5	»	7.0	1	21

Effect of osmolarity

During the initial stages of this investigation it was observed that the use of water as suspension medium gave a smaller yield of noradrenaline in the sediment than if isotonic solutions were used. The effect of varying osmolarity was therefore studied, using KCl, NaCl and $CaCl_2$ as suspension media. The primary suspension was prepared with water and the sediment resuspended in the electrolyte solutions. The total time of exposure of the granules to the different media was approximately 5 min of preparation and 30 min of centrifugation at pH 6.0—6.4 at a temperature of about +5° C.

Fig. 4 shows the effect of solutions of different tonicity, given in terms of osmolarity. In each series one sample was exposed to water as resuspension medium. The concentrations of isotonic solutions (0.3 osm) were approximated to 0.15 M for KCl and NaCl and 0.10 M for $CaCl_2$.

As seen in Fig. 4 maximal stability at pH 6.0—6.4 was observed in the region of 0.3 osmolarity, *i. e.* in isotonic solutions, while increased release occurred

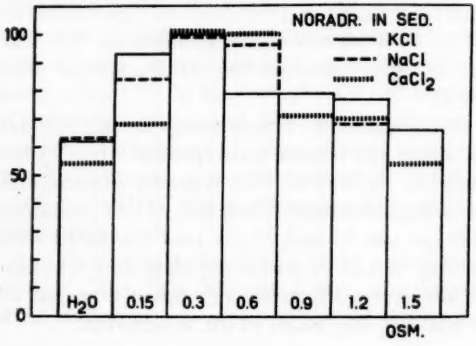

Fig. 4. Relative amount of noradrenaline in sediment after suspension in various media at different osmolarities. Exposure time 5 min. + 30 min centrifugation at + 4—5° C and pH 6.0—6.4.

Table III. Noradrenaline remaining in splenic nerve granules after incubation 30 min at + 20°C at pH 7.0 in 0.13 M potassium phosphate

Cetyl pyridinium bromide	10 mM	8.6 per cent of control
» » »	1 mM	17 » » » »
Sodium lauryl sulphonate	1 mM	7.8 » » » »

in hypertonic as well as hypotonic solutions. The relatively large release in strongly hypertonic solutions is notable. Even when water was used as suspension medium the amount of noradrenaline in the resedimented granules still was about 50—60 per cent of that in the controls in isotonic solution, indicating a moderate degree of lysis only.

Membrane active compounds

A considerable release of noradrenaline from the sedimented granules into the supernatant after addition of detergents, such as cetyl pyridinium bromide and sodium lauryl sulphonate, to the suspension media, has previously been observed (EULER 1958). In new experiments the resuspended granules were incubated with detergents and the amounts of noradrenaline present in the sediment and the supernatant measured. Both detergents in a concentration of 1 mM caused an almost complete loss of amine content in the transmitter granules in 30 min at + 20° C as shown in Table III.

Incubation for 30 min at + 20° C with saponin (MERCK) in concentrations of 1—30 mg/ml in the resuspended sediment at pH 7.0 in 0.075 M potassium phosphate did not alter the amounts of noradrenaline which were present in the sediment.

Ether in concentrations of 1 per cent in the primary suspension did not influence the yield of noradrenaline in the sediment.

Table IV. Influence of various electrolytes on the noradrenaline content in granules. Temp. + 5°C., Time 95 min., pH 6.8

Medium	Conc. M	Tonicity (Isotonic = 1)	Noradr. in sediment µg/g nerve
NaCl	0.15	1.0	1.09
KCl	0.15	1.0	0.99
$CaCl_2$	0.10	0.85	1.12
$MgCl_2$	0.10	1.0	1.16
K-phosphate	0.075	(0.6)	1.12
Na-acetate	0.15	1.0	1.03
H_2O			0.84

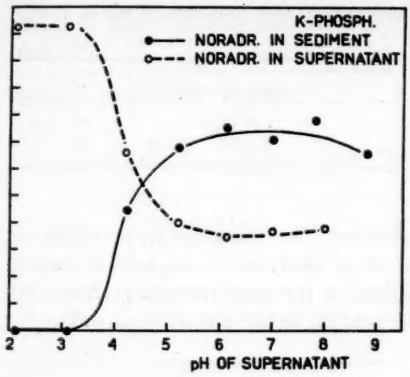

Fig. 5. Noradrenaline in sediment and supernatant from resuspended granules from bovine splenic nerves at different pH. Incubation medium 0.075 M potassium phosphate, time 30 min at + 20° C. Ordinate, arbitrary units from zero.

Electrolyte suspension media

A comparison of the effect of various cations and anions on the rate of release of noradrenaline from granules at low temperature did not reveal any significant differences as shown by Table IV. Nor did incubation for 30 min at + 22° C with the disodium salt of ethylene diamine tetraacetic acid cause any release of noradrenaline from the granules.

Effect of H^+ concentration

It has been observed previously that addition of acids to pH 3 or below caused a rapid release of the noradrenaline bound to the granules. In the present work the effects of pH on the release of the transmitter from the granular stores has been studied in more details. As suspension media water and various electrolytes were used to which acids and alkali were added to give the required pH. The following solutions were used: 0.15 M NaCl, 0.15 M KCl, 0.10 M $CaCl_2$, 0.10 M $MgCl_2$, 0.10 M K_2SO_4, 0.075 M K-phosphate and 0.15 M sodium acetate.

The granules showed good stability in the pH range of 5.3—8.8 with only small differences in the noradrenaline content when phosphate was used as suspension medium. The somewhat narrower maximal stability range in the other media (pH 6.0—8.5) may be due to the lack of buffering capacity of the media. For this reason the pH of the suspension medium may have been temporarily higher or lower than the final pH of the suspension on addition of acids or alkali. A partial release may therefore have occurred initially.

Complete loss of noradrenaline from the sediment was observed when the pH of the suspension was 3.5 and below. In the pH range 3.5—5.3 a partial release was observed during the experimental conditions prevailing.

Fig. 5 illustrates graphically the amounts of noradrenaline in the sediment and in the supernatant at different pH-values in the suspension. The figure

shows that the sum of these amounts is constant for each sample, indicating that all of the released amine is found in the supernatant.

A number of series were made with samples adjusted to pH 6.5—9.0 with narrower pH intervals in the region 7.0—8.0. Maximum stability was observed at pH 7.3—7.7 although the difference between the figures obtained in the region from pH 6.5—8.5 were small.

Effect of some autopharmacologically active agents

In some experiments the effect of various naturally occurring biologically active substances and some functionally related drugs have been studied after addition to a suspension of noradrenaline granules. After resuspension of the sediment in 0.075—0.13 M potassium phosphate and addition of the drug the suspension was incubated for 30 min at + 20° C. No effects were observed with acetylcholine, histamine, serotonin and GABA. Since serotonin seriously disturbed the analyses it had to be removed by selective adsorption of the noradrenaline on an alumina column. The dose ranges used were for acetylcholine 3.3—20 mg/ml, histamine 3—300 μg/ml, serotonin 0.2—10 mg/ml and for GABA 5—15 mg/ml. Nicotine 3—300 μg/ml was likewise without effect.

The actions of tyramine, dopamine, octopamine (kindly given to us by Prof. V. Erspamer) and phenylethylamine were studied in a special series the results of which have been previously reported (EULER and LISHAJKO 1960 a).

Discussion

The sediment obtained by high *g* centrifugation of a suspension prepared from adrenergic nerves by a simple squeezing procedure has proved to be useful for the study of a variety of factors on the storing ability of the microgranules. A number of observations support the assumption that these microstructures behave as if covered by a membrane having some properties in common with cell membranes. Thus the contents are readily released by addition of detergents or acids to a granule suspension. The loss of noradrenaline in hypertonic and hypotonic media and the maximal stability in isotonic solution likewise suggest a biomembrane.

That the particle bound noradrenaline is well protected is evident from the observations that manganese dioxide at pH 6 only inactivates the noradrenaline occurring free in solution. Furthermore the noradrenaline present in granules passes through an alumina column and can be released by addition of acid after the passage.

The mode of binding of the noradrenaline to the storage granules is still unknown. It has been shown by SCHÜMANN (1958) that nerve granules from adrenergic nerves contain ATP like the chromaffin cell granules (FALCK *et al.* 1956, BLASCHKO *et al.* 1956).

While the total noradrenaline present in the press juice from bovine splenic

nerves in our experiments has been on an average about 9 μg per g, the amount recovered in the sediment has been about 2—3μg per g, or 20—35 per cent of the total. Whether the amount found in the supernatant occurs in a free form or is loosely bound to granules or some other cell constituent is at present not possible to state. The granular fraction shows a high degree of stability at low temperature but readily gives off the noradrenaline at higher temperatures. Especially the rapid release observed in a preparation of granules in isotonic potassium phosphate at + 37° C is hardly compatible with a storage function under the conditions prevailing in the isolated system. The observations suggest rather that the system is in some way incomplete or disturbed, so as to enhance the releasing process.

Although no effects were observed with the different electrolyte solutions used in the suspension medium this of course does not exclude actions of cations and anions on the noradrenaline binding mechanism.

Acid reaction had a very strong releasing action below pH 4, apparently by splitting the bonds keeping the amines fixed to some constituent of the specific structures. Even at pH 4—5 this effect was observed although it was less marked.

A number of drugs have previously been found to cause a release of noradrenaline from the granular sediment, among them tyramine and reserpine (EULER and LISHAJKO 1960 a, b, SCHÜMANN 1960) and local anesthetics in high concentrations (EULER 1960). In view of the stimulating effect of acetylcholine and other compounds on chromaffin cells and postsynaptic adrenergic neurons it appeared of interest to study the effect of this compound on the granules. The absence of effect with this substance or with nicotine or serotonin seems to indicate that their cell stimulating effects are not effectuated by an action on the intracellular granules themselves but must be due to triggering an indirect releasing effect.

Some quantitative differences were noted between the nerve granules and the adrenal medullary granules with regard to the releasing effects of temperature, hypotonic solutions, freezing and thawing and detergents. Thus HILLARP and NILSON (1954) found only about 10 per cent release in 10 min at + 45° C in adrenal medullary granules (0.3 sucrose) while the nerve granules in our experiments had lost about 80 per cent of the noradrenaline in 10 min at 37° C (0.13 M potassium phosphate).

On the other hand freezing and thawing had a much stronger action on the adrenal medullary granules than on the nerve granules (about 70 per cent release as against 20—30 per cent).

With adrenal medullary granules HILLARP and NILSON (1954) found that lowering of the molarity of the suspension medium from 0.25 M to 0.15 M sucrose for 1 hour at + 3° C caused an almost complete release of the catecholamines in the granules. Even suspension in water released less than 50 per cent of the noradrenaline in the nerve granules in the same time, however.

The apparently larger effect of sodium lauryl sulphonate on nerve granules in comparison with medullary granules may be due partly to the smaller amount of material used in the experiments with nerve granules.

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Air Flow Patterns and Heat Transfer Within the Respiratory Tract

A new method for experimental studies on models

by

SVEN INGELSTEDT and NILS GUNNAR TOREMALM

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Abstract

INGELSTEDT, S. and N. G. TOREMALM. *Air flow patterns and heat transfer within the respiratory tract.* Acta physiol. scand. 1961. *51.* 204—217. — The authors have worked out an experimental method which makes it possible to determine air flow patterns in models of the respiratory tract. The method is based on the close relationship between heat transfer and pressure drop conditions which occur in a tube with a warm inner surface when air is blown or sucked through it. The air temperatures before entering and after leaving a tube of tracheal dimensions are determined and the heat transferred to the passing air is computed. Thus heat is used as a tracer. As a result of friction to flow, an aerodynamic boundary layer is built up. It is stated that the shape of this layer determines the actual degree of heat transfer between the airway mucous membranes and the respired air. The hitherto unknown biological importance of variations in the boundary layer within different parts of the respiratory tract is briefly discussed.

Variations in the type of air flow that occurs within the human respiratory tract have an influence not only on the mechanics of breathing but also, more directly, on the interchange of heat and moisture between the respired air and the mucous membranes. An intense wall contact is physiologic and valuable within some regions of the upper airways, e. g. the nose and the subglottic space (INGELSTEDT and TOREMALM 1960). During special conditions, e.g. oral breathing of room air with low relative humidity or breathing through a tracheal

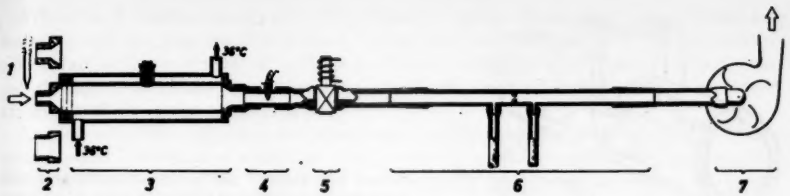

Fig. 1. The experimental device. 1. Thermocouple for measuring the actual room temperature. 2. The three variations of preconnected constriction. 3. Tracheal model with thermostat-regulated wall temperature. 4. Thermocouple for measuring the emerging air. 5. Electromagnetic valve. 6. Orifice meter. 7. Sucking fan regulated by an autotransformer.

stoma, however, an extreme degree of wall contact may be deleterious to the easily damaged ciliary epithelium (TOREMALM 1960, 1961). Flow patterns with reference to the upper respiratory tract, mainly the nose, have hitherto been studied only with pressure difference methods (SEMERAK 1958, TONNDORF 1958 and others) and with the aid of either dye-fluid (WEST and HUGE-JONES 1959) or smoke flow in model experiments (PROETZ 1953, BIRNMEYER 1959). The direct relation between pressure drop, flow patterns and degree of wall contact within the airways has not yet been thoroughly analyzed, which may be due to the lack of a sufficiently sensitive method for aerodynamic studies in short tubes (length less than 50 times the diameter) during intermittent flow and at physiological air flow rates.

In this paper a new method for such studies is described. It is based on the connection between aerodynamic and heat transfer phenomena appearing in a tube with warm walls when a gas — for example ordinary room air — is blown or sucked through it, whereby heat is used as tracer.

A tracheal model is tested in different manners well comparable with adult human airway conditions. The results are discussed from a physiological point of view, while the clinical applications are described in another paper (INGELSTEDT and TOREMALM 1960).

Method

A double-walled brass tube (length 140 mm, diameter 22 mm) was used as a tracheal model (Fig. 1; 3). The wall temperature was kept constant at 36° C with the aid of circulating water from a thermostat. Air was intermittently sucked through the system 16 times per minute by an electric fan at flow rates of 20—60 l/min. The air flow was regulated with an electromagnetic valve and was determined by an orifice meter. The tube was coned in one end, where a quick-responding micro-thermocouple was mounted (Fig. 1; 4). The thermocouple was made of constantan and nickel-chromium wires with a diameter of 0.03 mm and soldered end to end so that the joints had the same diameter as the wires. This instrument, which is illustrated in detail in Fig. 2, measured the temperature of the emerging air.

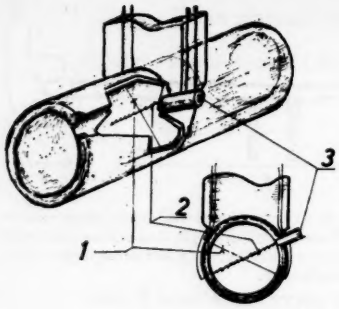

Fig. 2. The authors' micro-psychrometer in detail mounted in a double-walled plexiglass tube (inner diameter 8 mm). 1. The "dry bulb" thermocouple. (The "wet" bulb thermocouple (2) and the water supply channel (3) were not used in the present experiments.)

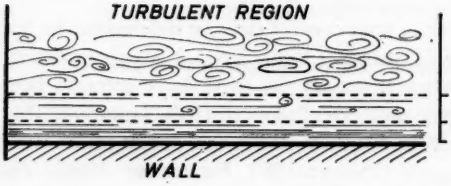

Fig. 3. The fully developed boundary layer during turbulent flow (A), the buffer layer (B) and the central turbulent air stream (C).

The room air temperature was kept fairly constant at 24° C, but minor changes could be measured with a second thermocouple (Fig. 1; 1) placed near the inlet for the air flow.

The cooled junctions of the thermocouples were immersed in ice (0° C). The potentials arising in the hot junctions were converted to a 50 c/p alternating current with the aid of Brown converters. The output voltages were recorded with a direct 2 channel ECG-apparatus (Mingograph, Type 230, ELEMA, Ltd., Stockholm). For computations of the results see KOCH *et al.* (1958) and Fig. 7.

Thus by recording the air temperature before entering (t_{in}) and after leaving the tube (t_{out}) the heat transfer was calculated as described below.

The following experimental series were performed:

1) Increasing degree of contraction with the aid of preconnected smooth circular constrictions (Fig. 1; 2) at the free end of the model.

2) Increase of entrance length with the aid of added preconnected tubes of the same diameter as the model.

3) Increase of entrance length and a constant contraction (diameter 8 mm) at the free end of the system.

4) Measurements of the pressure drop across a tube with the same dimensions as the model during steady state conditions whereby a sensitive alcohol manometer was used (20 mm alcohol column = 1 mm H_2O).

Fundamental properties of heat transfer.

There are three fundamental ways of heat transfer, i. e. conduction, convection and radiation. If the warming object is moist as well, heat may also be transferred by means of condensation or evaporation. (The latter is not discussed in this paper.)

It may be assumed that in the present experiments only convection and conduction are essential. If no air passes through the tube the enclosed air will soon attain the wall temperature i.e. through conduction and free convection.

When air is blown through the tube, however, special aerodynamic phenomena are involved and the heat transfer is more intense and more complicated (forced convection). Part of the incoming air adheres to the wall because of friction forces and a so-called laminar boundary layer is built up (Fig. 3). Between this and the central turbulent air stream there is also a buffer layer.

At increasing flow velocities the temperature of the outgoing air gradually decreases until a point is reached at which the temperature is sensibly constant and independent of further increment of the velocity. It was suggested by REYNOLDS already 1874 that this state is reached at the "critical" velocity point where a laminal flow is changed to a turbulent one.

In spite of the turbulent flow which normally exists in the upper respiratory tract (GAENSLER, MALONEY and BJÖRK 1952) such a laminar region is, however, still present near the wall. The thickness of this layer is mainly dependent on the air flow velocity.

Within the boundary layer and the structure of the wall heat is transferred by conduction, which is a relatively slow process as compared with heat convection. Therefore the thickness of the boundary layer practically determines the degree of heat transfer.

Theoretical calculations[1]

The heat transfer is characterized by the heat transfer coefficient (h) which is approximatively inversely proportional to the thickness of the boundary layer. It is defined by Newton's Law of heat transfer as follows:

$$Q = h \cdot F \cdot (t_{surface} - t_{room\ air}) \qquad (1)$$

where

Q = heat transfer per unit of time

F = surface area

$t_{surface}$ = surface temperature and

$t_{room\ air}$ = temperature of the surrounding air.

h = local heat transfer coefficient.

It may be observed that the heat transfer coefficient (h) varies along the tube. This variation depends on differences in structure and thickness of the boundary layer along the inner wall. As not only the boundary layer but also the main flow in the region of the tube inlet strongly depends on the contraction, it may be expected that also the heat transfer coefficient changes considerably at different degrees of inlet constriction. The tests entirely confirm this assumption. As far as we know, the heat transfer coefficient at different inlet constrictions has not yet been investigated and it may therefore be justified very briefly to make some suggestions as to this variation in the vicinity of the inlet.

In the very first part of the inlet there is no influence from disturbances and also no boundary layer. It is, however, necessary to assume a very high value of the heat transfer coefficient but it is of no importance whether this value tends towards infinity or whether it is finite. (See Fig. 4.)

Immediately after the constriction there are more or less stationary whirls and therefore the exchange of heat between the wall and the central part of the flow is small. It may be assumed that the influence of these whirls decreases further on and therefore the heat transfer coefficient increases.

When finally the laminar boundary layer is built up to a constant thickness the heat transfer coefficient becomes constant. It is difficult to predict if this final value is higher or lower than the value in the transient region, but it does not seem unreasonable to

[1] The authors thanks are due to Ass. Prof. C. Allander and G. Leide, Ph. D. for valuable assistance with this part of the investigation.

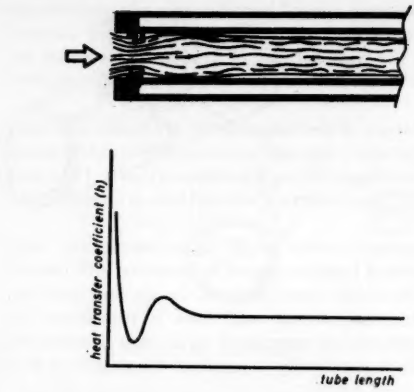

Fig. 4. The type of air flow in the original model (above) and in the model provided with an inlet constriction (below) in broad outline. In the middle the theoretical distribution of the heat transfer coefficient along the tube.

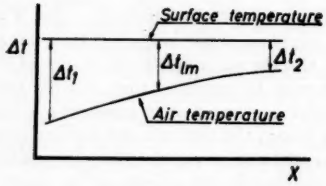

Fig. 5. The figure demonstrates how the logarithmic mean temperature difference is computed. $\triangle t_1$ = the temperature difference between tube wall and room air and $\triangle t_2$ = the temperature difference between tube wall and the outgoing air. $\triangle t_{lm}$ = logarithmic mean temperature difference.

assume that the variation is in accordance with Fig. 4. In the upper and lower part of this figure the flow is sketched in broad outline with and without inlet constrictions.

With regard to the choice of measurement method it is only possible to determine the mean value of the heat transfer coefficient along the tube. The size of the inlet constriction and the length of the tube must therefore influence this mean value.

Besides the heat transfer coefficient, the air temperature also varies along the tube. It is therefore necessary to introduce the well-known logarithmic-mean-temperature difference (GIEDT 1957) which is expressed by the following equation (See Fig. 5.):

$$Q = h_m \cdot F \cdot \frac{\Delta t_1 - \Delta t_2}{ln \frac{\Delta t_1}{\Delta t_2}} = h_m \cdot F \cdot \Delta t_{lm} \qquad (2)$$

where

Q = heat transfer per unit of time.
h_m = average heat transfer coefficient.
F = inner surface area of the tube.
Δt_1 = temperature difference between tube wall and room air.
Δt_2 = temperature difference between tube wall and emerging air.
Δt_{lm} = logarithmic-mean-temperature difference.*)

From the equation h_m may be solved

$$h_m = \frac{Q}{F \cdot \Delta t_{lm}} \tag{2^1}$$

The amount of heat transferred from the wall per unit of time (Q) is the same as that absorbed by the passing air according to the following equation

$$Q = c_p \cdot G \cdot (t_{out} - t_{in}) \tag{3}$$

where

Q = heat transfer per unit of time,
G = mass velocity (mass per unit of time),
$t_{out} - t_{in}$ = mean temperature increase of the air and
c_p = specific heat (at constant pressure).

The mass velocity is calculated as the product of flow rate (V) and mass density (ϱ) as follows:

$$G = V \cdot \varrho \tag{4}$$

Thus the average heat transfer coefficient (h_m) may be calculated from the equations 2^1, 3 and 4.

$$h_m = \frac{\varrho \cdot c_p}{F} \cdot V \cdot \frac{t_{out} - t_{in}}{\Delta t_{lm}} \tag{5}$$

In this equation the following units can be used.

ϱ in kg/m³
c_p in kcal/kg °C
F in m²
V in m³/h
t in °C.

Thus the average heat transfer coefficient (h_m) is expressed in kcal/m² h °C.

Example:

The model provided with a constriction (diameter 5 mm).

Tube inner surface is $0.94 \cdot 10^{-2}$ m², wall temperature 36° C, volume flow 60 l/min, room air (t_{in}) 24.5° C and outgoing air (t_{out}) 31.0° C.

Mass density 1.15 kg/m³ and specific heat = 0.24 kcal/kg °C

$$h_m = \frac{1.15 \cdot 0.24}{0.94 \cdot 10^{-2}} \cdot \frac{60 \cdot 60}{1{,}000} \cdot \frac{31 - 24.5}{7.8} = 88.0 \text{ kcal/m}^2 \text{ h °C}$$

*) The logarithmic-mean-temperature difference can easily be computed with the aid of a nomogram.

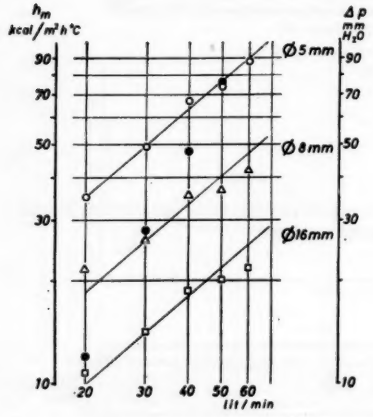

Fig. 6

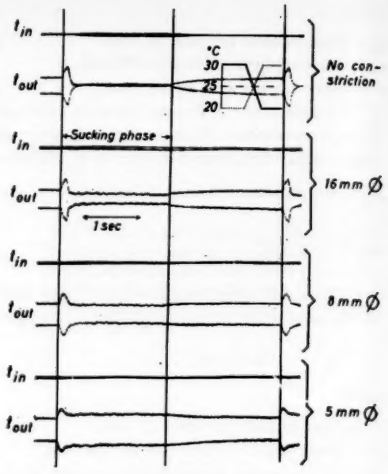

Fig. 7

Fig. 6. The computed heat transfer coefficients for the experiments with constrictions and the variations in air flow rate plotted on a logarithmic scale in order to obtain a straight line relationship. The theoretical slope for the heat transfer coefficient (0.76) is drawn. □ = diameter 16; △ = diameter 8 mm and O = diameter 5 mm. The pressure drop values (●) obtained during continuous flow with the 5 mm constriction, showing a slope closely related to the expected one which is 2.0.

Fig. 7. Temperature recordings of room air (t_{in}) and the outgoing air (t_{out}) at a flow rate of 30 l/min with increasing degree of constriction. Frequency 16/min. The sucking phase appears to the left in the recordings. The initial temperature rise is due to the considerable warming of the enclosed air during the silent phase (to the right in the recordings) when no sucking or blowing takes place. The diagram plotted to the right above shows how the recorded temperature curves are computed. The zero line is shifted to 25°C and thus a recorded temperature curve at 30° C has the same appearance as a curve at 20° C.

Results

A. *Tracheal model with increasing degree of inlet constriction.*

The following constriction diameters were used: 16, 8 and 5 mm. The constrictions correspond to a cross-sectional area of the human larynx, an ordinary tracheal cannula and a severe tracheal stenosis. The average heat transfer coefficients by increasing air flow rates are plotted on a double logarithmic scale in Fig. 6. Temperature recordings at an air flow rate of 30 l/min appear in Fig. 7. The temperature differences (t_{out}-t_{in}), the average heat transfer coefficient (h_m) and the amount of heat transferred per unit of time (Q) from the wall to the passing air for all experiments in this series are given in Table I (mean value of three determinations).

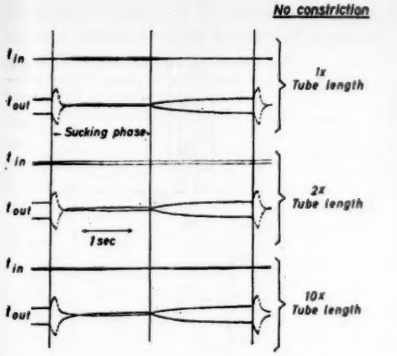

Fig. 8

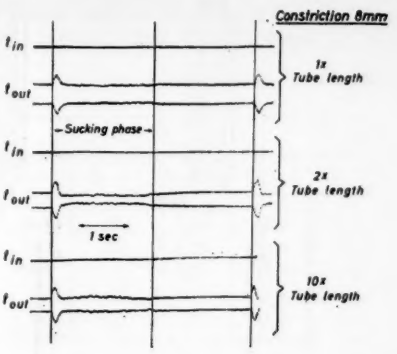

Fig. 9

Fig. 8. Temperature recordings at a flow rate of 30 l/min (frequency 16/min) with increasing degree of entrance length with the aid of preconnected tubes of the same diameter as the tracheal model.

Fig. 9. Temperature recordings at a flow rate of 30 l/min (frequency 16/min) with increasing degree of entrance length and with a constant constriction at the free end of the system (diameter 8 mm).

B. *Tracheal model with increasing degree of entrance length.*

A brass tube with the same diameter and with the same length (140 mm) as the model and another similar tube with a tenfold length (1,400 mm) were preconnected to the tracheal model. The temperature recordings at an air flow rate of 30 l /min appear in Fig. 8. The temperature differences, the average heat transfer coefficients, and the heat transferred to the passing air in this series appear in Table II (mean value of three determinations).

Table I. Temperature differences (t in °C), heat transfer coefficients (h_m in kcal/m² h °C) and heat transfer per unit of time (Q in kcal/h) with increasing degree of inlet constriction

Volume flow rate l/min	Constriction diameter									No constriction		
	16 mm			8 mm			5 mm					
	$t_{out}-t_{in}$	h_m	Q	$t_{out}-t_{in}$	h_m	Q	$t_{out}-t_{in}$	h_m	Q	$t_{out}-t_{in}$	h_m	Q
60	2,25	31.9	2,2	3,75	42.0	3,7	6,50	88,0	6.5	0,25	2,2	0.2
50	2,50	30,3	2,1	4,00	36,6	3.3	6,50	73,5	5.4	0,00	0	0
40	2,75	18.8	1,8	4,75	35,6	3.1	7,25	67,0	4,8	0.00	0	0
30	2,75	14,2	1,4	4,50	26,2	2,2	7,00	49.3	3.5	0,25	1,1	0,1
20	3,00	10,4	1,0	5.25	21,5	1,7	7,25	35.0	2,4	0.25	0,8	0.1

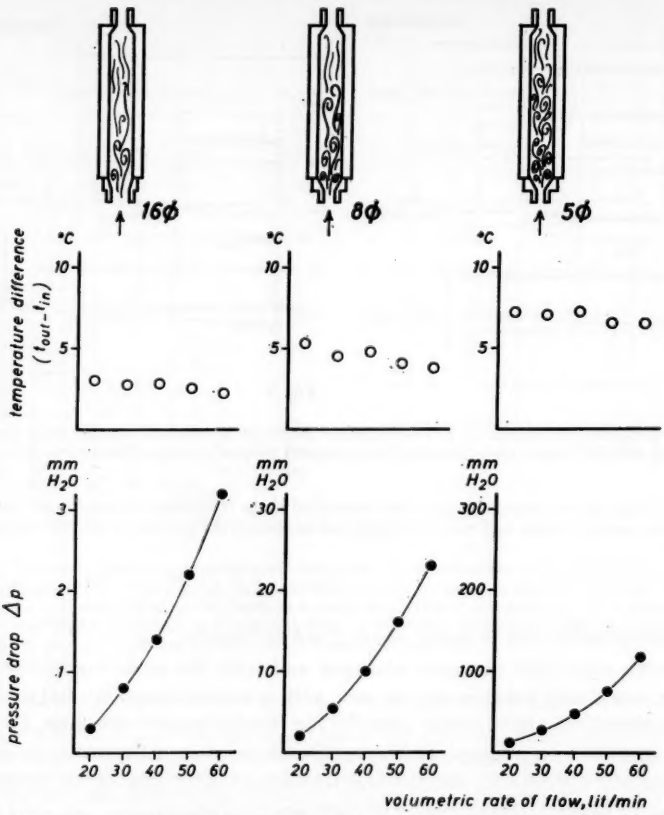

Fig. 10. The temperature increments at varying flow rates in experiments with constrictions appear in the diagrams above. Below the corresponding pressure drop values showing non-linearity (turbulence).

Table II. Temperature differences (t in °C), heat transfer coefficients (h_m in kcal/m² h °C) and heat transfer per unit of time (Q in kcal/h) with increasing degree of entrance length

Volume flow rate l/min	Tube length								
	× 1			× 2			× 10		
	t_{out}–t_{in}	h_m	Q	t_{out}–t_{in}	h_m	Q	t_{out}–t_{in}	h_m	Q
60	0.25	2,2	0.2	0.50	2,3	0.2	0.50	4.7	0.5
50	0.00	0	0	0.25	1,9	0.2	0.75	5,7	0.6
40	0,00	0	0	0,75	4,8	0,4	0,00	0	0
30	0.25	1.1	0,1	0.50	2,2	0,2	0.00	0	0
20	0.25	0,8	0.1	0.75	2.3	0.2	0,25	0	0

Table III. Temperature differences (t in °C), heat transfer coefficients (h_m in kcal/m² h °C) and heat transfer per unit of time (Q in kcal/h) with increasing degree of entrance length and with a constant inlet constriction

Volume flow rate l/min	Tube length								
	× 1			× 2			× 10		
	$t_{out}-t_{in}$	h_m	Q	$t_{out}-t_{in}$	h_m	Q	$t_{out}-t_{in}$	h_m	Q
60	3.75	42.0	3.7	2.00	20.4	2.0	2.50	27.0	2.5
50	4.00	36.6	3.3	2.25	19.4	1.9	2.75	24.7	2.3
40	4.75	35.6	3.1	2.25	13.8	1.3	3.00	21.8	2.0
30	4.50	26.2	2.2	2.25	11.8	1.2	3.00	16.3	1.5
20	5.25	21.5	1.7	2.50	8.6	0.8	3.25	11.7	1.1

C. *Tracheal model with increasing degree of entrance length and with a constant constriction (diameter 8 mm) at the free end of the system.*

The temperature recordings at an air flow rate of 30 l/min appear in Fig. 9. The temperature differences, the average heat transfer coefficients, and the heat transferred to the passing air during this experimental series are given in Table III (mean value of three determinations).

D. *Tracheal model with increasing degree of constriction compared with pressure drop determinations.*

The pressure drops across the model with preconnected constrictions were also determined (during steady state conditions) with a very sensitive alcohol manometer. These results appear in Fig. 10, where they are compared with the corresponding temperature differences (recorded during intermittent flow).

Discussion

The air flow within the human respiratory tract has hitherto mainly been considered from a purely mechanical point of view and little attention has been paid to air flow patterns and especially to the important direct contact that exists between the respired air and the mucous membranes. The latter conditions have not yet been investigated.

As regards air flow patterns in the nose, PROETZ (1953) has obtained very nice results with smoke in model experiments. Air flow patterns within the larynx have recently been studied in the same way by BIRNMEYER (1959) but only during continuous flow conditions. Furthermore none of these authors have reported what air flow rate they used. WEST and HUGE-JONES (1959) have also made some experimental observations on flow patterns in a lung model. They studied the distribution of a dye in streaming water, and in other experiments they injected argon with subsequent mass spectrometer analysis in gas samplings. As regards their experiments on inspiratory con-

ditions, however, no attention was paid to the influence of the upper respiratory tract.

None of the methods mentioned yields satisfactory information on the very essential wall contact and thereby on the heat exchange that takes place between the respired air and the mucous membranes in different parts of the human respiratory tract. Therefore, a more comprehensive knowledge concerning these problems is needed.

The present method — which is based on the close relation between pressure drop, flow rate and heat transfer in a tube with uniform wall temperature — makes such studies possible. The recorded temperatures of the air before entering and after leaving the model yields simultaneous information on air flow patterns and the degree of wall contact during intermittent flow. These conditions can never be determined by the use only of smoke, dye or pressure methods.

The degree of heat transferred to the air after passing through the experimental device is due to the shape of the boundary layer because heat is transferred by means of conduction within the boundary layer and by means of forced convection between this layer and the free, central air stream.

The chief object of the present study was

1. to test the method experimentally for comparison with theoretical calculations;

2. to evaluate the effect of variations in air flow patterns within the human trachea on the basis of experimental data. The results, however, also provide a reason for more general considerations regarding flow patterns in the whole respiratory tract.

Ad 1. The reliability of the method appears from Fig. 6 where the experimentally obtained slopes for the heat transfer coefficients and the pressure drops are seen to be almost identical with the expected theroretical ones.

Ad 2. Tracheal, bronchial and nasal aspects will be discussed one by one.

In the original model without constriction the critical velocity — the point at which a laminar flow becomes turbulent — was reached already at a flow rate of 30 l/min, which may be theoretically calculated. Therefore it may be concluded, in accordance with GAENSLER, MALONEY and BJÖRK (1952), that turbulence normally prevails within the adult trachea even during quiet breathing at rest in both respiratory phases. In addition it is obvious (see Table I and Fig. 7) that a turbulent type of flow may exist without any noteworthy direct contact between the wall and the airstream as the "inspired" room air passes nearly unchanged through the original model independently of air flow rates up to 60 l/min, i.e. at a velocity far above the critical one.

Tracheal conditions

It must be remembered that no part of the respiratory tract, not even the trachea, can be compared with a straight tube. This is due to the fact that the larynx has an influence on inspiratory and the main bronchi on expiratory

flow patterns. The former factor is not considered in the experiments made by WEST and HUGE-JONES. It is variations in the type of the entrance region itself and not in the entrance length that are essential. (See Table II and Fig 8.) In spite of a preconnected tube (length 1.4 m), hardly any heat exchange took place within the model. The constriction with 16 mm diameter has a cross-sectional area closely related to that of the adult human larynx during "inspiration". "Inspiration" through this constriction compared with "inspiration" without any constriction (Table I and Fig. 7) illustrates the increase in degree of wall contact distally to the constriction in the former case. The temperature of the emerging air increased by about 2—3° C which is in sharp contrast with the very small pressure differences obtained with a sensitive alcohol manometer. The direct comparison between heat transfer and pressure drop is illustrated in Fig. 10.

From this it may be concluded that the human larynx functions as a constriction in an aerodynamic sense.

With increasing degree of constriction — as in the case of tracheotomy and pathological processes in the airways (equivalent to the constriction diameters 8 and 5 mm) — the degree of wall contact also increases and the region distally to the constriction is always the most exposed one (see Fig. 3). Therefore, during inspiration the mucous membrane may be damaged by drying effects in that region. This is normally not the case as the inspired air is previously warmed and moistened to a very great extent in the nose. During prolonged oral breathing, however, this disadvantage may occur. During expiration no drying takes place but naturally the degree of heat and moisture recovery is influenced by constricting processes. It must be observed that the temperature differences in this experimental series were not affected by variations in flow rates from 20 to 60 l/min but naturally the amount of heat transferred to the air is trebled, which results in increased demands on the mucous membrane.

BRISCOE, FORSTER and COMROE (1954) have made some experiments with inhalation of helium and oxygen. They found that gas portions considerably less than the normal dead space were able to penetrate the dead space and to reach the alveolar level, which suggests a laminar cone front. This statement, however, need not necessarily be irreconcilable with the present ones, as these authors used 80 per cent helium, which has a far lower density than air and thus decreases or eliminates the turbulence.

Bronchial conditions

In their experiments on a lung model WEST and HUGE-JONES (1959) found that there was a laminar type of flow in the trachea during the inspiratory phase up to a flow rate of 10 l of air per minute. At high flow rates (40 l/min) eddying was seen at the main carina and other bifurcations. From our experiments with preconnected straight tubes and a constant constriction (8 mm) at the free end of the system it appears (see Table III and Fig. 9) that the turbulence that

arises distally to the constriction extended throughout a length of 1.4 m. Therefore it seems to be possible that, in the human trachea (larynx included), turbulence exists even at a flow rate of 20—30 l/min and that the unsteadiness in the air flow proceeds below the main carina as the tracheal cross-sectional area is nearly equal to that of the main bronchi (JESSEPH and MERENDINO 1957). This is however of little importance for the mucous membranes in this region since the inspired air is conditioned to about 80 per cent (INGELSTEDT 1956) already at the level of the larynx.

Nasal conditions

A very intense wall contact and a relatively thin boundary layer must be expected to occur in the nose during normal conditions. This assumption naturally presupposes a turbulent type of air flow in both directions but probably to a greater extent during the inspiratory phase. After studying the investigations of SEMERAK and of SEEBOHM and HAMILTON, TONNDORF (1958) concluded that nasal air flow during normal breathing is at the verge of eddying or becoming turbulent. Pressure drop determinations across only one nasal cavity are unphysiological but on the other hand, when they are made across both nasal cavities, they are very small and thus unreliable. Furthermore the following informations make likely a turbulent flow in the nose: a) irregular inner configuration, b) narrower cross-sectional area in the vestibuli nasi as compared with that of the present model, where turbulence appeared already at a flow rate of 30 l/min and c) rapid warming of inspired air as shown by INGELSTEDT and TOREMALM (1960). (During breathing in a cold chamber at —12° C the inspired air had a temperature of + 25°C already when it reached the epipharynx.)

Conclusions

From the present experiments and considerations the following conclusions can be drawn:

1. Air flow studies in the respiratory tract are of great interest if they also yield information on the degree of contact between the mucous membrane and the respired air.

2. The shape of the boundary layer — built up through friction to flow — is very important and determines the degree of wall contact and hereby also the degree of heat transfer to the inspired air.

3. A turbulent type of flow exists during both respiratory phases in the nose, trachea and main bronchia during normal conditions.

4. From a functional point of view the human larynx is the narrowest region of the entire respiratory tract and functions as a constriction in an aerodynamic sense. The inspired air from nose and mouth is mixed in the subglottic space, where consequently the boundary layer is disturbed. This may be of importance at prolonged oral respiration and in pathological conditions.

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On the Origin of Lithocholic and Ursodeoxycholic Acids in Man

Bile Acids and Steroids 106

By

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Abstract

HELLSTRÖM, K. and J. SJÖVALL. *On the origin of lithocholic and ursodeoxycholic acids in man.* Acta physiol. scand. 1961. *51.* 218—223. — Chenodeoxycholic acid-24-^{14} C was given perorally to two healthy persons and intravenously to one patient having a bile fistula. In the normal subjects bile was collected from the duodenum every morning for three days. Only a minor part of the chenodeoxycholic acid had been metabolized. About 3 per cent of the activity recovered from the healthy subjects was present as lithocholic acid. Ursodeoxycholic acid accounted for 2 per cent of the activity in one of these subjects. None of these acids were found in the bile fistula patient. Unidentified labelled compounds more polar than chenodeoxycholic acid were present in all bile samples.

Lithocholic and ursodeoxycholic acids have been isolated in small amounts from human bile and lithocholic acid has also been found in human feces (SOBOTKA 1937, SJÖVALL 1959, MOSETTIG *et al.* 1958). In rats and rabbits these acids can be formed from chenodeoxycholic acid (SAMUELSSON 1959, MAHOWALD *et al.* 1958, NORMAN *et al.* 1960, HELLSTRÖM *et al.* 1960). Since chenodeoxycholic acid is one of the main bile acids in human bile, knowledge of its metabolism is important in quantitative studies of bile acid turnover. In order to see whether lithocholic and ursodeoxycholic acids can be formed in man during the enterohepatic circulation of chenodeoxycholic acid we have given chenodeoxycholic acid-24-^{14} C to two healthy subjects and one patient with a bile fistula. The results are presented in this paper.

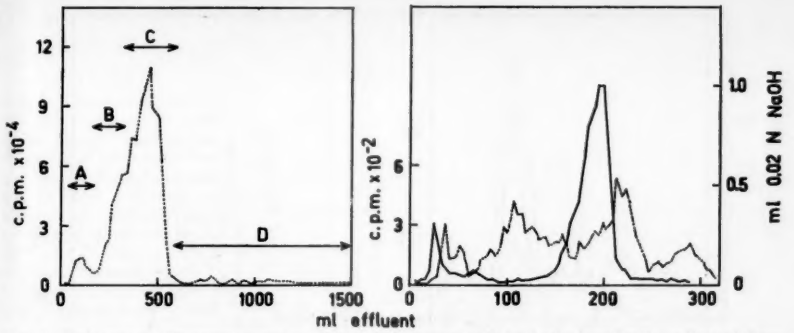

Fig. 1. Left curve: Chromatography of labelled compounds in hydrolyzed bile collected from the duodenum after administration of labelled chenodeoxycholic acid to a healthy subject. Column: 45 g hydrophobic Supercel, phase system F 1. Right curve: Rechromatography of peak A (left curve) with phase system C 1. Column: 9 g Supercel. Solid line: Titration. Dotted lines: Radioactivity.

Experimental

Chenodeoxycholic acid-24-^{14} C with a specific activity of 10 μC per mg was synthesized according to BERGSTRÖM *et al.* (1953). Ursodeoxycholic acid, m. p. 202—203, was synthesized according to SAMUELSSON (1960). Two young healthy female subjects were given 3.7 μC of the labelled acid *per os*. Fasting bile was collected every morning for three days. The samples were obtained from the duodenum through a polyvinyl tube after the intravenous injection of cholecystokinin-pancreozymin (LINDSTEDT 1957) and the collection was continued as long as there was a flow of dark coloured bile.

In addition to the two normal subjects the labelled acid was given to one female patient, 59 years old. A T-tube had been inserted into the common bile duct following the removal of gallstones. Her liver function tests were normal and no bile pigments were present in the feces at the time of the experiment. Bile was collected for 12 hours after the intravenous injection of the labelled acid.

Extraction and chromatography. The bile samples were added to about 10 volumes of ethanol. After filtration and evaporation of the ethanol the bile acids were hydrolyzed in 1 N NaOH at 120° C for 6 hours. They were then extracted with ether after acidification of the aqueous solution. Reversed phase chromatography was performed as previously described (SJÖVALL 1953, NORMAN 1953).

The following solvents were used.

Phase system	Moving phase (ml)	Stationary phase (ml)
C 1	Methanol-water 150 : 150	Chloroform-isooctanol 15 : 15
F 1	Methanol-water 165 : 135	Chloroform-heptane 45 : 5
F 2	Methanol-water 180 : 120	Chloroform-heptane 45 : 5

Hydrophobic Hyflo Supercel was used as the inert support for the stationary phase.

Radioactivity was determined by counting of an infinitely thin layer on aluminium planchets in a Frieseke-Hoepfner (FH 51) methane gas-flow counter.

Results

Normal subjects. A total of 14 and 26 per cent of the radioactivity was recovered from the duodenum of the respective subjects after the administration of the labelled chenodeoxycholic acid. Chromatography of the hydrolyzed bile with

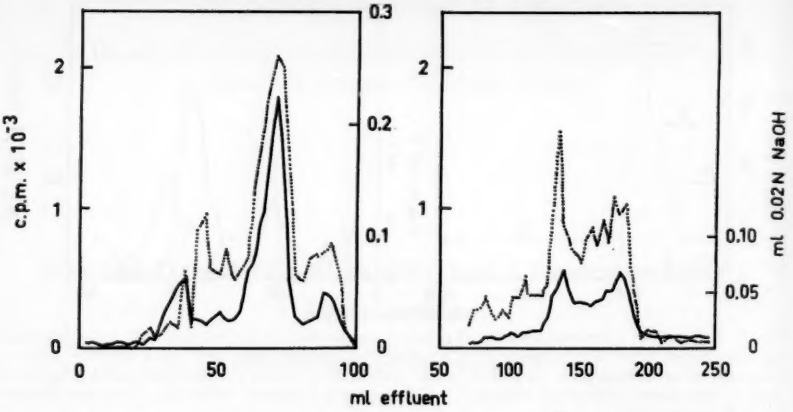

Fig. 2. Left curve: Second rechromatography of band B (Fig. 1, left curve) together with unlabelled ursodeoxycholic acid. Column: 9 g Supercel, phase system F 1. Right curve: Rechromatography of band D (Fig. 1, left curve) together with unlabelled lithocholic acid (see results). Column: 9 g Supercel, phase system F 2. Solid lines: Titration. Dotted lines: Radioactivity.

phase system F 1 showed that only 9 per cent of the chenodeoxycholic acid had been transformed in both subjects. The result of the analysis of the bile from one subject is shown in Fig. 1 (left curve). As seen, a small peak of radioactivity appears with the front (A). The main peak has the same elution characteristics as pure standard chenodeoxycholic acid. Ursodeoxycholic acid is eluted from the columns slightly faster than chenodeoxycholic acid and if a small amount of labelled ursodeoxycholic acid were present it would be expected to elute with the first part of the large chenodeoxycholic acid peak. This peak was therefore divided into two parts which were rechromatographed separately (B and C). The fractions collected after the chenodeoxycholic acid peak were combined (D) for further chromatographic analysis. Column chromatographic analysis of the bile from the second subject was similar to the first and the fractions were treated in the same way.

Band A (Fig. 1) was rechromatographed with phase system C 1 and the result was identical for both subjects (Fig. 1, right curve). No peak of radioactivity was found with the titration peak of the cholic acid originally present in the bile (Fig. 1, right curve, 150—210 ml effluent). At least three radioactive compounds not identical with cholic acid were present.

Band B (Fig. 1) was rechromatographed with phase system F 1 and the fractions thought to contain ursodeoxycholic acid were combined. These fractions were again rechromatographed after addition of unlabelled ursodeoxycholic acid. No labelled compound was eluted with the ursodeoxycholic acid peak in one of the subjects. However, labelled material was found in the

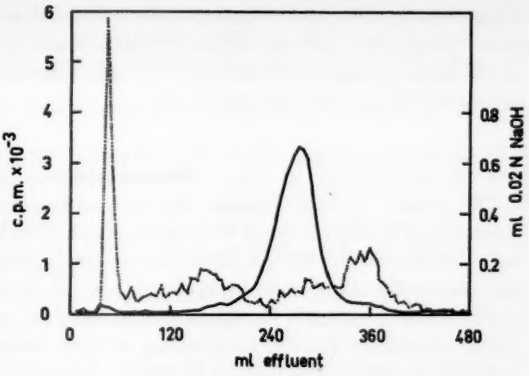

Fig. 3. Chromatography of labelled compounds more polar than chenodeoxycholic acid obtained from the bile fistula patient during 12 hours following the intravenous administration of chenodeoxycholic acid-24-^{14}C. Column: 13.5 g Supercel, phase system C 1. Solid line: Titration. Dotted line: Radioactivity.

ursodeoxycholic acid peak of the second subject as shown in Fig. 2 (left curve). The small peaks of radioactivity on both sides of the ursodeoxycholic acid peak (60—80 ml effluent) were due to the incomplete removal of chenodeoxycholic acid and the more polar compounds present in band A (Fig. 1). The identity of the labelled compound with ursodeoxycholic acid was established by isotope dilution.

Most of the radioactivity both in band B and band C (Fig. 1) coincided with the titration peak of the chenodeoxycholic and deoxycholic acids from the bile. It was therefore regarded as unchanged chenodeoxycholic acid.

The combined fractions designated D (Fig. 1) were rechromatographed with phase system F 2 after addition of unlabelled lithocholic acid. Radioactive peaks coinciding with the titration peak of the carrier were found in both experiments. One of the chromatograms is shown in Fig. 2 (right curve). Unfortunately crystallization of lithocholic acid on the column resulted in the appearance of a double peak. This is not commonly seen with bile acids other than lithocholic acid. The identity of the labelled compound with lithocholic acid was established by isotope dilution.

Bile fistula patient. To avoid influences of the intestinal flora on the metabolism of the chenodeoxycholic acid, the labelled acid was given intravenously to the patient with a T-tube in the common bile duct. Although the feces were acholic, passage of bile into the duodenum could not be completely excluded. Therefore only the bile excreted during the first 12 hours after the injection was used for the analysis of the labelled compounds formed. During this time about 25 per cent of the activity injected was recovered. Chromatography of the hydrolyzed bile showed that about 15 per cent of the activity was present as compounds more polar than chenodeoxycholic acid. The rechromatography of these compounds with phase system C 1 is shown in Fig. 3. The chromatogram is similar to the one obtained with the bile of the normal subjects (Fig.

1, right curve). No peak of radioactivity coincided with the cholic acid titration peak (about 200—300 ml effluent). Rechromatography of the fractions that would have contained ursodeoxycholic and lithocholic acids respectively, did not reveal the presence of these acids.

Discussion

The results obtained indicate that chenodeoxycholic acid is less extensively transformed in man than in rats, mice, pigs and rabbits. In rats and mice the 3α, 6β, 7α- and 3α, 6β, 7β-trihydroxycholanic acids are the main metabolites and these acids are formed from chenodeoxycholic acid in the liver (HSIA *et al.* 1958, SAMUELSSON 1959, DANIELSSON *et al.* 1959). In pigs the intestinal flora is of great importance for the metabolism of chenodeoxycholic acid. This acid is 6α-hydroxylated in the liver to hyocholic acid which is then transformed into hyodeoxycholic acid by intestinal microorganisms (BERGSTRÖM *et al.* 1959). This transformation involves the removal of a 7α-hydroxyl group in analogy with the formation of deoxycholic acid from cholic acid and of lithocholic acid from chenodeoxycholic acid (LINDSTEDT *et al.* 1957, BERGSTRÖM *et al.* 1959, NORMAN *et al.* 1960). Removal of the 7α-hydroxyl group from cholic and chenodeoxycholic acids appears to be a dominant reaction in the bile acid metabolism of the rabbit (LINDSTEDT *et al.* 1957, HELLSTRÖM *et al.* 1960).

In the two normal human subjects studied bile was collected every morning for three days after the administration of labelled chenodeoxycholic acid. The combined bile samples from each of the two subjects contained about 3 per cent of the radioactivity as lithocholic acid. This acid was not to be found in the patient having a bile fistula indicating that the lithocholic acid is formed by intestinal microorganisms as is the case in the rat (NORMAN *et al.* 1960). Acids behaving similar to, but not identical with cholic acid were found in all three subjects. The chromatograms of these compounds were similar and it is therefore possible that they were formed in the liver. Two per cent of the radioactivity in the bile of one of the normal subjects was present as ursodeoxycholic acid. This acid was not found in the other subjects but it is known that ursodeoxycholic acid cannot be found in all human bile samples (SJÖVALL 1959).

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Acta physiol. scand. 1961. 51. 224—229

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Tyramine Effects on Catechol Amine Release from Spleen and Adrenals in the Cat

By

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Abstract

STJÄRNE, L. *Tyramine effects on catechol amine release from spleen and adrenals in the cat.* Acta physiol. scand. 1961. *51.* 224—229. — Recent studies on the sympathomimetic effect of tyramine suggest that this substance may act by liberating noradrenaline. In the present experiments direct bioassay of the venous effluents from the spleen and the adrenals of the cat demonstrated that the noradrenaline level in splenic vein blood was elevated by tyramine, whereas in the adrenal venous blood both the adrenaline and noradrenaline levels were either unchanged or depressed. The discrepancy between the effects of tyramine on these two systems is discussed. It is concluded that in the spleen tyramine may act either by releasing noradrenaline from the intraaxonal storage granules, or by making extragranular noradrenaline, bound to some structure probably in close relation to the axone, available to the receptors.

The observations of BURN and TAINTER (1931), that tyramine lost ist dilator effect on the cat's iris after denervation by removal of the superior cervical ganglion one or two weeks previously, and of BURN (1932), that the vasoconstrictor effect of tyramine in the cat's foreleg was reduced or abolished after similar removal of the stellate ganglion, suggested that the sympathomimetic action of tyramine is dependent on intact sympathetic innervation.

In 1953 FLECKENSTEIN proposed as one possible interpretation that tyramine, as well as other sympathomimetic amines having two hydrogen atoms at the β-carbon in the side chain and either no hydroxyl group in the ring or one such group in para position, might exert its action by releasing noradrenaline.

The recent finding, that the vasoconstrictor action of tyramine is strongly reduced after the sympathetically innervated organs have been emptied of most of their catecholamines by reserpine treatment (CARLSSON *et al.* 1957), as well as the observation, that its normal action can be restored by noradrenaline and to some extent by adrenaline infusions intravenously, have been regarded as further evidence that tyramine acts by releasing noradrenaline (BURN and RAND 1958).

In vitro experiments have demonstrated that tyramine liberates catechol amines from isolated storage granules, both from splenic nerves (EULER and LISHAJKO 1960, SCHÜMANN 1960) and adrenals (SCHÜMANN 1960).

The present experiments have been designed to study the action of tyramine on the adrenal medulla and the spleen of the cat, by collection of the venous effluent and direct determination of its catechol amine content, following tyramine administration.

Methods

Cats weighing 2.5—5.0 kg and anesthetized with Nembutal 35 mg/kg i.p., were prepared for collection of adrenal vein blood according to the method of SCHAPIRO and STJÄRNE (1958). Heparin was given as anticoagulant, 4 mg/kg i.v. initially and half this dose each hour.

The adrenal vein blood was collected in graduated centrifuge tubes. After centrifugation at 1000 × *g* for 5—10 min, hematocrit and volume were determined and the plasma withdrawn and stored in a freeze-box for subsequent bioassay.

In order to study the relationship of vasoconstriction in the spleen to "overflow" of noradrenaline in the venous effluent, the following procedure was used: A clamp was placed across the middle part of the spleen, and one half of it was perfused with arterial blood from the left femoral artery of the same cat by a Sigma motor pump, giving a constant flow rate. The arterial blood pressure in this circuit, in the present report referred to as splenic arterial pressure, as well as the systemic arterial pressure, heart rate and respiration were recorded on a Grass polygraph, using commercial transducer equipment. The venous effluent from the spleen was collected intermittently according to the same principles as those used for the adrenal vein blood. The main vein was ligated 2—4 cm from the organ and a polyethylene catheter was introduced into the vein, pointing towards the spleen. During collection periods the blood was diverted through a T-tube into graduated centrifuge tubes, whereas in the intervals it was returned to the cat via the right femoral vein. After centrifugation, hematocrit and volume determination and separation from the cells, the plasma was kept in a freeze-box for later catechol amine determination. For differential assay of adrenaline and noradrenaline in the adrenal vein blood, the cat's blood pressure and the hen's rectal caecum were used. Pressor activity in splenic vein blood was determined on the blood pressure of the cat, premedicated according to KÄRKI (1956). No effort was made to distinguish between adrenaline and noradrenaline in the splenic effluent since it is known that more than 90 procent is noradrenaline (PEART 1949).

The effect of tyramine on the secretion from the adrenals was studied before and after denervation by splanchnicotomy.

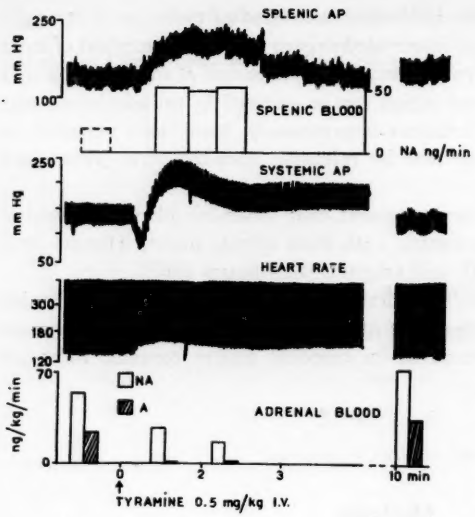

Fig. 1. The effect of 0.5 mg/kg tyramine intravenously on splenic arterial pressure, noradrenaline output from the spleen, systemic arterial pressure, heart rate and adrenaline and noradrenaline secretion from the innervated adrenal.

Results

Tyramine 0.5—1.0 mg/kg i. v. in the cat caused a powerful pressor response, occasionally preceded by a depressor phase, and a sometimes extreme tachycardia, after pretreatment with atropine, 6 mg/kg intraperitoneally.

The cardiovascular responses to repeated doses of tyramine showed a rapid decrease in cats treated with reserpine, 3 mg/kg i. v. 1—2 hours earlier. The initial response was partially restored after i. v. infusion of 15 μg/kg/min of noradrenaline for 5 min, after the blood pressure had returned to the preinfusion level.

The adrenal medullary secretion showed no increase during the peak of the pressor response to tyramine. On the contrary, it was clearly depressed in most cats with intact adrenal innervation. This depression was not seen after adrenal denervation by splanchnicotomy. After the peak of the cardiovascular response, blood pressure and heart rate often failed to return to the resting level for about 10 min. During this phase there was a slight increase of adrenaline and noradrenaline in the adrenal vein blood.

A further attempt to stimulate adrenal medullary secretion with tyramine was made by perfusing an isolated cattle adrenal according to the venous perfusion method of *Hechter et al.* (1953), with oxygenated, heparinized blood from the same animal. No definite increase in the catechol amine content of the effluent was seen as a result of an infusion of tyramine, 10 mg/min for 10 min.

The resting noradrenaline level in the splenic vein blood was close to the

threshold of the present assay method and is represented in Fig. 1 as being less than 2.5 ng/ml. During the vasoconstrictor phase in the spleen following tyramine, pressor activity in splenic vein blood rose to 6—8 ng/ml.

Discussion

The increase in pressor activity in the splenic vein blood in the cat caused by tyramine — as a sign of an increased "overflow" of noradrenaline — confirms the hypothesis of FLECKENSTEIN (1953), that tyramine exerts its sympathomimetic action by causing a local release of noradrenaline.

Similar observations have recently been published by LOCKETT and EAKINS (1960). They found that tyramine caused an increase both in the adrenaline and the noradrenaline concentrations of plasma from the aorta but not from the vena cava of adrenalectomized, hexamethoniumtreated cats. Their conclusion is that tyramine liberates both adrenaline and noradrenaline either from some stores in the arterial walls, or from the red cells.

The adrenals do not contribute to the immediate cardiovascular effects of tyramine, in the dosage used. This is in agreement with the results reported by STRÖMBLAD (1960), obtained with a different technique. The inhibition of the adrenal medullary secretion during the peak of the hypertensive response seen in most of the experiments did not occur after adrenal denervation and is thus probably reflex in origin. This is in accordance with the experiments reported by SCHAEPDRYVER (1959), who found inhibition of the adrenal medullary secretion when the isolated perfused carotid sinus of the dog was exposed to a local elevation of pressure, relative to the systemic pressure.

The small increase in adrenaline and noradrenaline secretion from the adrenals seen after the peak of the pressor response possibly indicates some contribution from the adrenals to the persistence of a slight elevation of blood pressure and heart rate, as compared to resting levels. However, this increase may well be a reflexly mediated "rebound" phenomenon, since it does not seem to occur after adrenal denervation.

The absence of any definite sign of catechol amine release from the isolated perfused cattle adrenal, even with a very high dose of tyramine, may be regarded as additional evidence that the intact adrenal medulla is very insensitive to the amine liberating effect of this agent.

The present results indicate that the effect reported by SCHÜMANN (1960), that tyramine incubation accelerates the spontaneous release of catechol amines from isolated storage granules from cattle adrenals does not operate on the intact organ.

On the other hand, the liberation by tyramine of noradrenaline in vitro from isolated storage granules from splenic nerves in cattle (EULER and LISHAJKO 1960, SCHÜMANN 1960) may well explain the release of noradrenaline from the cat's spleen, obtained in the present experiments with tyramine.

The striking discrepancy between the catechol amine releasing effect of tyramine in the spleen and the adrenals might possibly be explained in terms of differences in accessibility to tyramine of the storage granules, in the two types of organ. Or else there may be a difference in the sensitivity to tyramine of storage granules from nerve tissue and chromaffin cells, as actually indicated by the differences in threshold concentrations for the releasing effect of tyramine observed in vitro (EULER and LISHAJKO 1960, SCHÜMANN 1960).

However, the fact that even a very high dose of tyramine does not release any appreciable amount of catechol amines from the adrenal, whereas a small dose is an effective releaser of noradrenaline from the spleen, suggests a different explanation.

The restoration by an i.v. infusion of noradrenaline of the gradually decreasing pressor response to tyramine after reserpine treatment observed in the present investigation, as well as several similar observations in the literature, notably by BURN and RAND (1958), indicate the existence of a storage mechanism for circulating catechol amines.

In contrast to the intraaxonal storage granules, this store appears to be characterized by a very limited uptake capacity, judging from the fact that uptake has been possible to demonstrate only by the sensitive radioactive isotope methods (AXELROD 1960). Yet it is functionally important, since it restores the effects, not only of tyramine but of sympathetic nerve stimulation as well (ROSELL 1959, BURN and RAND 1960), after these have been abolished by reserpine treatment. The implication of this appears to be that the postulated storage structure is closely related to the nerve fibre, possibly even located on the surface of the axone.

If it is assumed that the sympathomimetic action of tyramine consists in releasing noradrenaline from a storage structure of the abovementioned type, related to the postganglionic sympathetic neurone but not to chromaffin cells, the discrepancy between the effects of tyramine on the spleen and the adrenals would seem to be explained.

The observation of tachyphylaxis in the blood pressure responses to tyramine less than two hours after reserpine, as well as the partial restoration of the tyramine response by a noradrenaline infusion are in agreement with the findings of BURN and RAND (1958). In normal cats the blood pressure response to tyramine remains unchanged for hours, whereas in the experiments described here a rapid decline was seen during minutes after reserpine. Thus reserpine evidently interferes with the resynthesis and/or storage capacity for noradrenaline.

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Acta physiol. scand. 1961. 51. 230—238

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Respiration and State of Wakefulness in Normals, Studied by Spirography, Capnography and EEG

A preliminary report

By

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Received 28 October 1960

Abstract

BÜLOW, K. and D. H. INGVAR. *Respiration and state of wakefulness, studied by spirography, capnography and EEG.* Acta physiol. scand. 1961. *51*. 230—238. — In sixteen normal subjects the relation between the state of wakefulness and the respiration was studied by recording continuously the ventilation (spirography), the alveolar carbon dioxide tension (capnography) and the EEG. Records were obtained in the resting state awake, in drowsiness and, in nine of the subjects, in sleep and during arousal. It was confirmed that ventilation decreases and that the alveolar carbon dioxide tension increases in sleep (MAGNUSSEN 1944). When the EEG showed a stable pattern of *e. g.* alpha-activity, or sleep waves, the breath to breath variations in ventilation and alveolar carbon dioxide tension were small at respective levels. There was in all the sixteen cases a close correlation in time between sudden changes in EEG and changes in the respiratory variables recorded. The findings suggest a close functional relationship between the respiratory centers of the brain stem and the reticular activating system which supposedly controls the cortical state of excitability.

In his monograph on respiration in sleep, MAGNUSSEN (1944) clearly demonstrated the difference between wakefulness and sleep as to average respiratory minute volume and alveolar carbon dioxide tension. In sleep, average ventilation is generally decreased and there is an increased carbon dioxide tension in the alveolar air as compared with the wakeful state. Furthermore, in sleep there is usually a characteristic change in form of the individual breath as recorded in a respiratory valve at the mouthpiece by a pressure sensitive device.

Alveolar carbon dioxide concentration has been further studied by ROBIN et al. (1958) and BIRCHFIELD, SIEKER and HEYMAN (1959) who like MAGNUSSEN (1944) found a decreased sensitivity to carbon dioxide during sleep in normals. REED and KELLOGG (1958) investigated respiration in sleeping subjects at sea level and after acclimatisation to high altitude. When falling asleep there was a definite increase in threshold value of ventilatory response to carbon dioxide at both altitudes.

In the present investigation we have recorded the EEG continuously in normal subjects which spontaneously passed through the stages of wakefulness, drowsiness, sleep and arousal. At the same time, respiratory functions were registered by means of continuous spirometry as well as continuous infra-red analysis of the carbon dioxide content of the ventilatory air (capnography). The "end-plateau" value of each expiration was taken to represent the alveolar carbon dioxide tension (P_{ACO_2}). In this way it was possible to study the gross time relationships between changes in the EEG and in respiration when changes in wakefulness took place. It will be shown that sudden EEG changes during shifts in the state of wakefulness were accompanied by simultaneous rapid alterations in ventilation as well as in alveolar carbon dioxide tension. The results suggest an intimate functional relationship between those parts of the brain stem which determine the cortical excitatory state (as reflected in the EEG), i. e. the so called reticular activating system (MAGOUN 1958) and those commonly designated as the "respiratory centers".

Material and Methods

The investigation was carried out in the mornings in sixteen normal subjects (ten females and six males), aged 22 to 60, most of these being nightworkers at the hospital and accustomed to going to bed at the time of the recording period. All persons had undergone a routine EEG, as well as a chest radiography and a complete analysis of respiratory functions (including determination of the different lung volumes and capacities, the resting minute volume, the maximal voluntary ventilation, the oxygen consumption and the distribution of the ventilatory air and its relation to the pulmonary blood flow). All subjects showed normal EEG:s and pulmonary conditions and there were no signs of bodily or mental disorder.

Recording was carried out in a dark quiet room with a suitably constant temperature. EEG electrodes were mounted symmetrically over the parieto-occipital regions on both sides of the scalp, 3 cm from the midline, and a continuous "bipolar" record was obtained in two leads by means of an ELEMA EEG-recorder (time constant 0.1 sec). In some cases the electrocardiogram was recorded by two electrodes placed on the right and left arm.

The subject to be investigated was lying comfortably in a reclining position and was allowed to breathe through a rubber mouthpiece, the extraoral part of which was glued air-tight with Mastix to the upper and lower lip. A nose-clip was also used. From a thin metal tube in the mouthpiece a small part of the ventilatory air was continuously carried to an infra-red absorption analyzer for carbon dioxide (Capnograph, Godart) by means of suction.

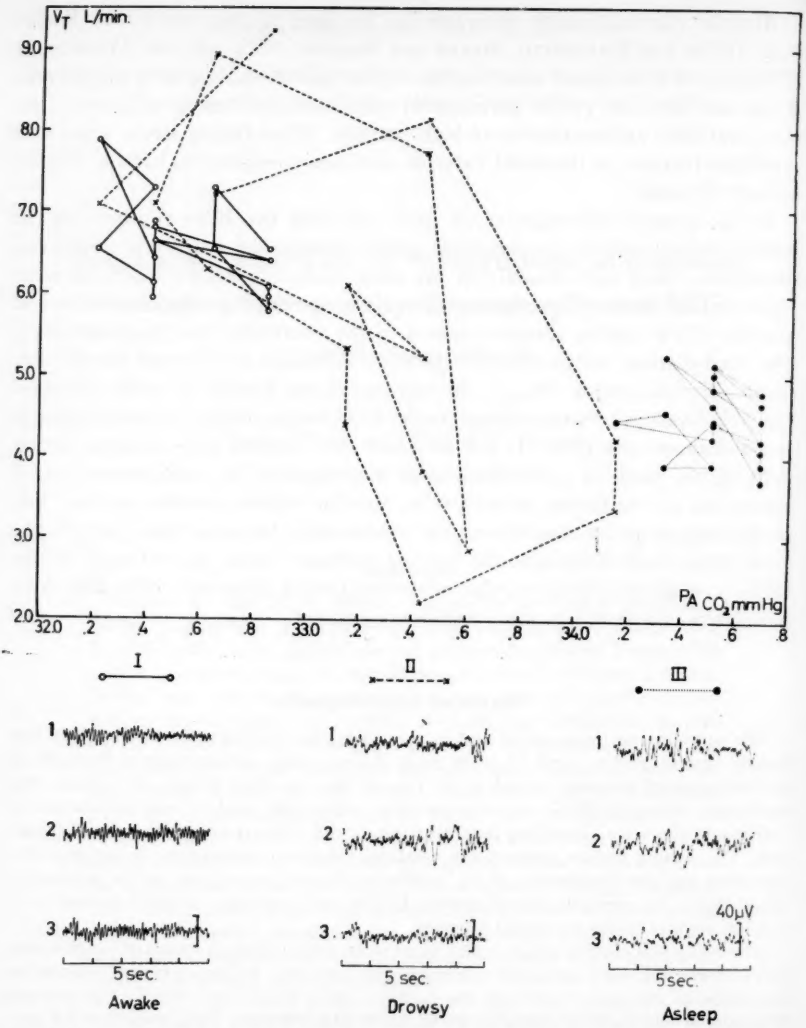

Fig. 1. Normal female subject, 26 years old. Diagram showing a consecutive breath by breath analysis of ventilatory flow ($\dot{V}_T$) and alveolar "end-plateau" carbon dioxide tension ($P_{A\,CO_2}$). Three periods of each 77 sec were analyzed when the subject was I awake at rest (lines drawn in full), II drowsy (dashed lines), and III asleep (dotted lines). (In the curve II four breaths were excluded in which the expiratory volume was too small to obtain an alveolar plateau.) Note small variations in $\dot{V}_T$ and $P_{A\,CO_2}$ when awake (I) and asleep (III), the latter group of values indicating lower ventilation and increased P_{CO_2}. Note further the scattered values pertaining to drowsiness (II).
The EEG samples were taken at the beginning (1), the middle (2) and at the end (3) of each period. In drowsiness the alpha periods in the EEG correlated to higher ventilation and lower carbon dioxide tension. The non-alpha periods with some theta and slow wave activity, on the other hand, correlated to lower ventilation and higher $P_{A\,CO_2}$.

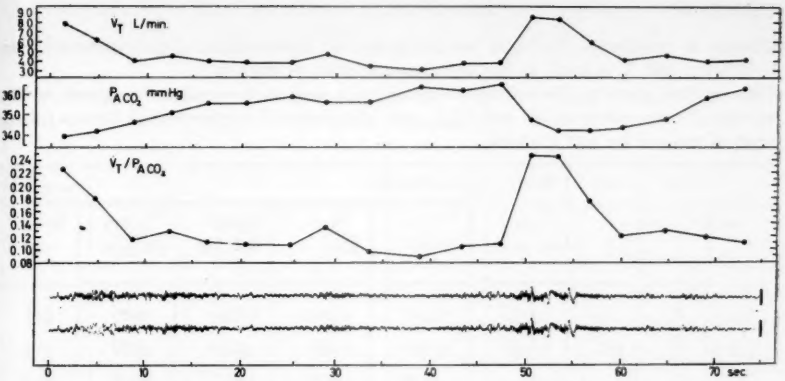

Fig. 2. Normal female subject, 20 years old. Diagram of consecutive breath by breath variations of ventilation and P_{ACO_2} in relation to EEG during a typical period of drowsiness with fluctuating awareness. The quotient $\dot{V}_T/P_{ACO_2}$ indicates "sensitivity" or ventilatory response to carbon dioxide. See text for further description.

The capnograph used permitted a detection of very small changes in P_{CO_2} (accuracy $\pm$ 0.3 mm Hg within the range 30 — 45 mm Hg) and reacted fast (90 % response time 0.1 sec). It was connected to a writing unit (Omniascriptor, Godart) with a linear frequency response up to 100 cycles per sec. The end-expiratory carbon dioxide tension (converted to STPD, i. e. standard temperature and pressure of dry gas) for any single breath was measured from the end-plateau value of the record with an accuracy of $\pm$ 0.2 mm Hg. In normals at rest the end-expiratory P_{CO_2} may be considered as representative of the mean alveolar carbon dioxide tension (P_{ACO_2}) (COLLIER, AFFELDT and FARR 1955, GÖPFERT and HENNEBERG 1956). The air sucked through the capnograph was shunted to the spirometer (Instrumenta), which was connected to the mouthpiece by a two-way valve. A closed spirometric system with soda lime absorber and gas circulation fan was used. The added respiratory dead space of this apparatus was 15 ml.

During the whole recording period which generally lasted from two to four hours the oxygen consumed was continuously replaced through a flow meter. The oxygen in the system was maintained at the level of 40 per cent in air. Thus, any stimulation from low arterial oxygen tension upon ventilation was avoided.

A continuous ink record was obtained from the spirometer. The ventilatory movements were also recorded on the same paper as the EEG by means of resistance variations in a potentiometer mounted on a moving part of the spirometer. Thus, individual breaths and their relations to sudden changes in the EEG could easily be studied. From the spirographic records the ventilatory flow ($\dot{V}_T$; converted to BTPS, i. e. body temperature and atmospheric pressure of gas, saturated with water vapor) during single breaths was calculated.

Different states of wakefulness were classified from the EEG according to the system of LOOMIS, HARVEY and HOBART (1938), GIBBS and GIBBS (1951) and from JUNG (1953). As a rule, the subjects were awake initially with a more or less continuous "modulated" alpha activity in the EEG (stage A). A period of drowsiness then followed with fluctuating awareness during which the alpha activity disappeared in the EEG and there developed some theta activity (stage B). In nine cases light sleep with slow waves and sleep spindles

Table I.

Changes in ventilation ($\dot{V}_T$), alveolar carbon dioxide tension ($P_{A\ CO_2}$) and quotient $\dot{V}_T$ per B (drowsy) and C (asleep) in five individuals selected at random.
The columns marked "Index" show the relative change in ventilatory response to CO_2 as unit. All the values of $\dot{V}_T$ and $P_{A_{CO_2}}$ are the means of ten consequtive breaths (except stage of wakefulness was changed.)

	Sex	Age	BSA m^2	A (Awake)				
				$\dot{V}_T$ l/min	$\frac{\dot{V}_T}{BSA}$	P_{ACO_2} mm Hg	$\frac{\dot{V}_{T/BSA}}{P_{ACO_2}}$	Index
1	F	28	1.75	5.40	3.09	32.4	0.95	1
2	F	22	1.70	4.95	2.91	32.3	0.90	1
3 a	F	25	1.98	5.16	2.61	37.2	0.70	1
b				[1]5.00	[1]2.53	37.9	0.67	1
4	M	49	2.00	8.15	4.08	32.3	1.26	1
5	F	60	1.53	4.46	2.92	36.5	0.80	1

(stage C) and in a few cases deeper sleep with high voltage delta activity (stages D and E) then followed. At the end of each recording period the sleeping subjects were usually aroused by being talked to and the ensuing changes were studied in the EEG and the spirogram.

In five of the subjects the quotient $\dot{V}_T/P_{ACO_2}$ (as the mean of ten consecutive breaths) was calculated for different states of wakefulness in order to get an index of the relative change in ventilatory response to carbon dioxide between the different states of wakefulness.

Results

A complete series of recordings including wakefulness, drowsiness and sleep was obtained in 9 of the 16 subjects studied. In a few cases periodic breathing was recorded intermittently at a certain level of wakefulness. Such findings are not dealt with in the present report, which is concerned with those respiratory changes which in a more or less complete fashion could be recorded in all subjects which went to sleep and showed the characteristic EEG patterns.

The main finding of the present investigation, the close correlation between the EEG pattern and the ventilatory state was demonstrated both at steady states and during sudden or gradual changes in wakefulness.

At steady state, i.e. when the EEG pattern was homogenous for longer periods, the ventilation ($\dot{V}_T$ in l/min) and the alveolar carbon dioxide tension (P_{ACO_2} in mm Hg) varied very little. In diagrammatic form this fact is demonstrated in Fig. 1 in which three equally long periods during wakefulness, drowsiness and sleep were analyzed in one subject. It is seen that the $\dot{V}_T/P_{ACO_2}$ relationship in wakefulness and in sleep — with continuous alpha activity or

m² body surface area (BSA) / $P_{A_{CO_2}}$ during three stages of wakefulness; A (awake),

("CO_2 sensitivity") when the corresponding quotient $\dot{V}_T/BSA$ / $P_{A_{CO_2}}$ of stage A is taken for the values marked with ¹, where there were only five consecutive breaths before the

B (Drowsy)				C (Asleep)			
$\dot{V}_T$ l/min	$P_{A_{CO_2}}$ mm Hg	$\frac{\dot{V}_{T/BSA}}{P_{A_{CO_2}}}$	Index	$\dot{V}_T$ l/min	$P_{A_{CO_2}}$ mm Hg	$\frac{\dot{V}_{T/BSA}}{P_{A_{CO_2}}}$	Index
4.68	33.6	0.79	0.83	4.18	33.2	0.72	0.75
3.72	35.2	0.62	0.69	3.58	35.8	0.59	0.65
4.67	36.7	0.64	0.91	—	—	—	—
4.52	36.8	0.62	0.92	—	—	—	—
7.52	33.1	1.14	0.90	6.35	34.3	0.93	0.73
3.98	37.0	0.70	0.88	3.56	38.2	0.61	0.76

sleep-pattern in the EEG respectively — remained within narrow limits (Fig. 1; circles and dots respectively). In contrast, the same relationship in drowsiness with its fluctuating EEG pattern showed large variations (Fig. 1, crosses).

In the diagram of Fig. 1 it is also seen that during sleep the ventilation diminished and the $P_{A_{CO_2}}$ increased, i. e. the points in the diagram shifted down to the right. This finding confirms fully the observations of MAGNUSSEN (1944) and the other authors mentioned above, who studied respiration in sleep.

In five subjects more complete data were obtained and the ventilatory response to carbon dioxide (the "carbon dioxide sensitivity"; i.e. the $\dot{V}_T/P_{A_{CO_2}}$ quotient) could then be compared during different periods of equal length showing EEG patterns A, B and C, representing wakefulness, drowsiness and sleep respectively. In Table I it is seen that if the "sensitivity" during stage A was taken as unit, then "sensitivity" in stage B varied between 0.69 and 0.92 and in stage C between 0.65 and 0.76. In later experiments the "carbon dioxide sensitivity" was investigated more systematically by administering carbon dioxide in increasing doses (by rebreathing) at different levels of wakefulness. The results of these sensitivity tests will be the object of a later publication.

During changes from wakefulness to sleep, or the opposite, during arousal, the ventilatory changes were also found to be very rapid. In Fig. 2 and 3 two examples are given of the concomitant EEG and respiratory changes when a subject went to sleep and was aroused.

In Fig. 2 the rising $P_{A_{CO_2}}$ during successive alphaloss and theta-slow wave appearance is clearly seen. The ventilation in this case showed an initial more

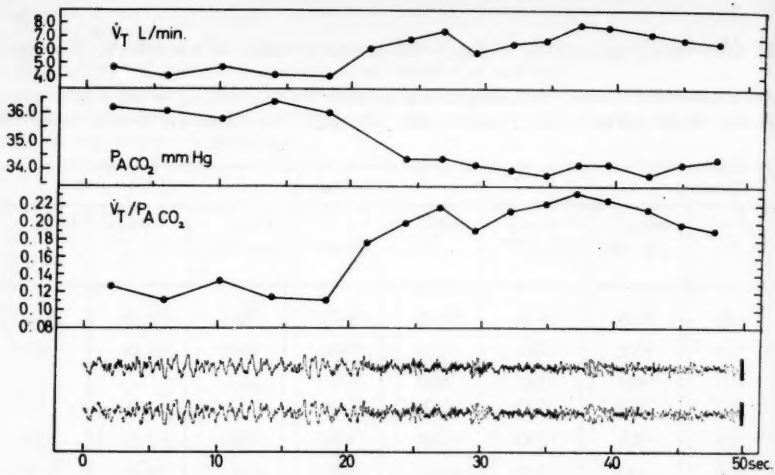

Fig. 3. Normal female subject, 20 years old. Diagram of ventilation and P_{ACO_2} during arousal (at 20th second). See text.

sudden decrease. Such a lag in the P_{ACO_2} during the initial minute of sleep onset was often seen. When, in this case, alpha activity appeared again for about 10 sec, there were simultaneous transient changes in ventilation and P_{ACO_2}. Effects of arousal are shown in Fig. 3.

Both from Fig. 1 and 2 it is evident that the state of drowsiness represents a non-steady state in which the ventilation varied very closely with the EEG changes between the wakefulness and the sleep type.

Discussion

In the analysis of the results we have accepted the well established opinion (COLLIER et al. 1955, ASMUSSEN and NIELSEN 1957, and others), that in normal humans at rest (with intact ventilation-perfusion relationship and mechanics of the lung) there is a close parallelism between the arterial and the mean alveolar carbon dioxide tension. It was also mentioned above that the expiratory end-plateau P_{CO_2} measured in normals at rest might be regarded as representative of the mean alveolar P_{CO_2}. Hence, it would seem justified to conclude that under the present experimental conditions, i. e. at rest in steady state, the alveolar end-plateau P_{CO_2} recorded provides an index of changes in the P_{CO_2} of the arterial blood, which may affect the respiratory centers. This index should, however, be considered a relative measurement since it cannot be concluded that the alveolo-arterial P_{CO_2} and the tension exerting its effects in the brain stem are identical.

In the present breath by breath analyses the total ventilation, not the alveolar ventilation, has been related to the P_{ACO_2}. During regular breathing at rest the tidal volume varies closely in parallel with the effective alveolar volume (GRAY, GRODINS and CARTER 1956, and others). Since, furthermore, the variations in ventilatory frequency at rest are small, the changes in total ventilation may be considered to vary closely in parallel with the effective alveolar ventilation. The ventilatory response of the respiratory centers to carbon dioxide may therefore be measured from the total ventilation at least when only moderate changes in ventilation are involved.

In view of the assumptions made in the aforegoing section we have interpreted the present results in the following way.

In general, the earlier findings of MAGNUSSEN (1944) and others have been confirmed. In sleep there is a decreased ventilatory response to carbon dioxide as compared to when awake. In addition, however, by the use of EEG, it has been possible to show a close time relationship between transient, sudden, or gradual, shifts in wakefulness and changes in ventilation.

It should be emphasized, however, that the present technique has a limited time resolution since it is obviously limited to an intermittent breath by breath analysis. Hence there is no possibility from the present results to establish in detail whether the respiratory or the EEG changes occurred exactly simultaneously or whether one or the other was leading.

Although the present analysis does not include a second to second analysis of the ventilation — P_{ACO_2} — EEG relation, the time relations shown nevertheless seem evident enough. The marked general synchrony between ventilatory and EEG changes in the records which was seen in all subjects would seem to have a wider implication. Such a synchrony suggests, in fact, a close functional relationship between centers in the brain stem which regulate the respiration and those commonly considered to control the cortical state of excitability, as this is reflected in the EEG, centers commonly referred to under the term the reticular activating system of the brain stem (MAGOUN 1958). A similar opinion has previously been expressed by BONVALLET, HUGELIN and DELL (1955), who studied experimentally the influence of carbon dioxide upon the reticular system of the brain stem.

The general conclusion reached above would imply that patients with some disturbance of their wakefulness regulation with ensuing EEG changes would also show respiratory irregularities. It is therefore of interest to note that SIEKER, HEYMAN and BIRCHFIELD (1960) reported depressed respiratory function with retention of carbon dioxide in eight cases of narcolepsy. Furthermore, POOLE (1960) recently reported a close correlation between changes in respiratory functions and the episodic EEG abnormalities which occurred in two cases of subacute encephalitis. In another series we studied respiration and EEG with the technique described in a group of patients with "heart neurosis" and typical sigh breathing (Säufzeratmung) (i.e. patients with severe subjective

heart symptoms without any demonstrable organic heart disease). In such cases there were also episodic transient subtle EEG changes which were correlated to the sighing breaths. The result of this investigation will be published later.

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Distribution and Concentration of Adrenaline and Noradrenaline in the Adrenal Medulla of the Rat Following Reserpine-Induced Depletion

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Abstract

ERÄNKÖ, O. and V. HOPSU. *Distribution and concentration of adrenaline and noradrenaline in the adrenal medulla of the rat following reserpine-induced depletion.* Acta physiol. scand. 1961. *51.* 239—246. — Adrenaline and noradrenaline concentrations in the adrenal medulla were determined after chromatographic separation by fluorometry. The chromaffin reaction, the iodate reaction and formalin-induced fluorescence were used as histochemical reactions and their intensities in the sections were quantitatively measured by microphotometry. Four mg/kg of reserpine daily for 4 days caused a pronounced loss of adrenaline and noradrenaline and recovery took about 3 weeks. During the recovery adrenaline and noradrenaline concentrations increased proportionally. The chemical and the histochemical measurements gave identical results. There was no evidence of local resynthesis of noradrenaline in excess of that of adrenaline. The results are at variance with observations reported earlier from other laboratories.

A large number of studies have been published demonstrating a pronounced loss of catecholamines from the adrenal medulla after administration of reserpine (CARLSSON and HILLARP 1956, ERÄNKÖ and HOPSU 1958, CAMANNI, LOSANA and MOLINATTI 1958, CALLINGHAM and MANN 1958 a, b, COUPLAND 1959, and others). The recovery of the medullary hormones after such depletion has been much less studied; we have found only the papers by CALLINGHAM and MANN (1958a, b) and COUPLAND (1959) to deal with it. CALLINGHAM and MANN (1958a, b) found that the noradrenaline content of the adrenals in

a few days not only returned to normal but increased greatly above it, while the adrenaline content reached the control value much more slowly; they studied only the content of catecholamines. COUPLAND (1959) used also histochemical reactions to investigate the distributions of adrenaline and noradrenaline; he reported that during the recovery phase noradrenaline is present in greater than normal amounts in those regions of the medulla which are normally concerned with storing adrenaline. Both CALLINGHAM and MANN (1958a) and COUPLAND (1959) concluded that methylation of noradrenaline is the rate-limiting factor in the synthesis of adrenaline.

In the present report observations are described in which both chemical and quantitative histochemical methods were used for studying the changes in the adrenomedullary catecholamines after reserpine administration.

Material and Methods

Experimental

Adult albino rats were used. In preliminary experiments, varying single doses of reserpine were injected and the depletion and recovery of the catecholamines was examined using only histochemical techniques (chromaffin reaction, iodate reaction, formalin-induced fluorescence). Similar experiments were then made using both histochemical and chemical methods of analysis.

The main experiments were carried out by using, first, 2 mg of reserpine per kg body weight subcutaneously daily for 3 consecutive days. This is twice the dosage used by CALLINGHAM and MANN (1958a). The daily dose was in later work increased to 4 mg/kg, and the rats were injected for 4 days. The animals were killed 1—24 days after the last injection by decapitation, together with about the same number of similar but untreated control rats. The adrenals were thereupon removed and halved. One of the 4 halves was used for chemical determinations, the remaining 3 ones for histochemical studies.

Chemical methods

The piece of adrenal was frozen on the microtome table and fresh sections were cut at 50 μ. The sections were frozen-dried and pieces of pure medulla dissected out. These pieces were weighed to the nearest μg, the catecholamines were separated by paper chromatography in a phenol-hydrochloric acid system and the separated amines were then determined fluorometrically. The fluorometric method used was essentially that used earlier in this laboratory (ERÄNKÖ 1954) but a higher concentration of ascorbic acid was used as proposed by EULER and FLODING (1955). This method is based on oxidation by ferricyanide; it was first described by EHRLÉN.

Chromaffin reaction

Adrenal halves were immersed in a mixture of 1 volume of 35 % formaldehyde and 19 volumes of 3.5 % potassium dichromate for 24 hours and, subsequently, in 3.5 % formaldehyde for another 24 hours. Frozen sections were then cut at 50 μ; these were mounted in glycerol.

Iodate reaction

Adrenal halves were immersed in a saturated solution of potassium iodate (HILLARP and HÖKFELT 1955) for 24 hours and, subsequently, in 3.5 % formaldehyde for 24 hours. Sections cut at 50 μ were mounted in glycerol.

Formalin-induced fluorescence

Adrenal halves were fixed for 3—4 hours in a solution containing 1 volume of 35 % formaldehyde, 6 volumes of 2 % calcium chloride and 3 volumes of distilled water. Sections cut at 50 μ were mounted in glycerol and studied for fluorescence.

Quantitative measurement of histochemical reactions

The optical densities of the chromaffin reaction and the iodate reaction were measured with a microphotometer. Details of the apparatus and the method have been described elsewhere (ERÄNKÖ and RÄISÄNEN 1960). Measurements were carried out in two sections of each adrenal half, taking density readings from different parts of the medulla. In sections treated for the iodate reaction, the densities of the medullary background, consisting of the almost iodate-negative adrenaline-containing cells, and of the specific islets composed of the iodate-positive noradrenaline-containing cells were recorded separately. The density measurements were made against a blank area on the slide, setting the photometer reading at zero density (100 % transmission). The results are expressed in terms of density (*i. e.*, the negative logarithm of the transmission).

The fluorescence intensity was measured using photographic photometry (for details see ERÄNKÖ and RÄISÄNEN 1960). Readings were taken from both the weakly fluorescent medullary background and the fluorescent medullary islets, which correspond to the iodate-negative and -positive parts of the medulla, respectively. The intensity of the medullary fluorescence varied for methodological reasons somewhat from day to day (see ERÄNKÖ and RÄISÄNEN 1960); therefore, the fluorescence intensity was expressed in terms of per cent of the mean intensity of the corresponding region of the adrenal medulla of those control animals killed together with the injected animals.

Results

In the preliminary experiments with a single dose of reserpine, variable loss of medullary substances giving the three histochemical reactions was observed. With lower doses only some medullary cells lost their chromaffin reaction, while the normally iodate-positive and fluorescent medullary cell groups exhibited a total loss or a marked decrease in the intensity of these two histochemical reactions. This suggests in agreement with earlier observations (ERÄNKÖ and HOPSU 1958) that the effect was limited mainly to the noradrenaline-containing cells.

With larger single doses of reserpine and three daily injections of 2 mg/kg, the chromaffin reaction was negative or of decreased intensity in larger areas of the adrenal medulla, showing that the treatment had also caused a loss of adrenaline. In all these experiments the medullary concentrations of both

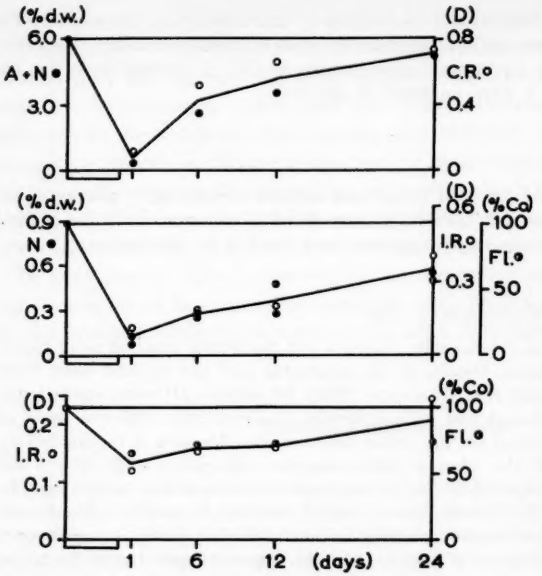

Fig. 1. Effect of reserpine on catecholamines of the adrenal medulla.

The injection period is indicated by a white rectangle below each graph (4 mg/kg daily for 4 days). The time scale is days after the last injection.

Top graph: Concentration of both catecholamines (A + N; full circles), expressed as % of dry weight of medulla, and intensity of the chromaffin reaction (C. R.; open circles), expressed in terms of optical density. Each circle represents the mean of 6—8 animals. The line has been drawn between the mean chemical and histochemical values.

Center graph: Concentration of noradrenaline (N; full circles), % of dry medullary weight; intensities of the iodate reaction (I. R.; open circles) in terms of optical density and of the formalin induced fluorescence (Fl.; half-filled circles), % of the corresponding mean control value. The intensities of the iodate reaction and the formalin-induced fluorescence were measured from the noradrenaline-containing cell islets.

Bottom graph: Intensities of the iodate reaction and the formalin-induced fluorescene in the medullary "background", *i. e.* adrenaline-storing cells. Symbols as in the center graph. Note that fluorescence is expressed as % of the corresponding (*i. e.* background) mean control value; therefore the fluorescence scales in this and the center graph are not comparable.

adrenaline and noradrenaline returned to normal within 4 days after the last injection. The chromaffin reaction, the iodate reaction and the intensity of the formalin-induced fluorescence were then similar of intensity and distribution as the corresponding reactions in the controls.

After daily injections of 4 mg/kg of reserpine during 4 consecutive days, pronounced effects were obtained. The results of the chemical analyses and of the quantitative measurements of the histochemical reactions are presented in Fig. 1. Each circle in each graph represents a mean value of a group of 6—8 animals, excepting the controls whose number was 26. The total number of animals in this experiment was 53. The injection period is indicated by a white rectangle below the base line. The scales of each graph have been adjusted so as to set the mean control values at the same height, which makes easier the comparison of the catecholamine concentrations and the intensities of the histochemical reactions.

The top diagram of Fig. 1 shows that there was an almost total loss of both

catecholamines 1 day after the last injection and that there was also a corresponding loss in the intensity of the chromaffin reaction, which actually was entirely negative in most sections, the residual loss of light being due to refraction rather than absorption (*cf.* ERÄNKÖ and RÄISÄNEN 1960).

Both the catecholamine concentration and the intensity of the chromaffin reaction returned slowly to normal. When these variables were expressed as % of the mean control value, and these relative values were compared, it was found that there was no statistically significant difference between the chemical and the histochemical data, although the mean density of the chromaffin reaction was somewhat higher 6 and 12 days after the last injection than the catecholamine concentration.

The center diagram of Fig. 1 shows corresponding changes in the noradrenaline concentration of the medulla and in the intensities of the iodate reaction and the formalin-induced fluorescence in the specific noradrenaline-containing medullary cell islets. There is a surprisingly good agreement between these variables, and it is clearly evident that in this experiment no increase over the control value in the medullary concentration of noradrenaline occurred during the restitution period. On the contrary, noradrenaline concentration tended to return more slowly towards normal than the total catecholamine concentration.

The bottom diagram illustrates the changes in the intensities of the iodate reaction and of the formalin-induced fluorescence in the medullary background, which consists of cells normally storing adrenaline. It is to be noted that the density and fluorescence scales are not the same as those in the center diagram, since again the mean control values have been placed at the same height from the zero line as in the two diagrams above.

No significant differences were found between the relative changes of these two reactions in the background, both showing first a decrease and then a slow return to normal. The relative decrease was smaller in these histochemical reactions than in the noradrenaline concentration but this is to a great part due to the facts that scattering results in a non-specific loss of light even in practically colourless sections and that there was some residual non-specific fluorescence in the medullary cells even after practically total loss of noradrenaline. There was no increase in the intensity of these histochemical reactions over the mean control value at any stage of the experiment.

Discussion

In our experiments 2 mg/kg of reserpine daily for three days caused a transient loss of catecholamines from the adrenal medulla but their concentration returned to normal within 4 days. Four consecutive daily injections of 4 mg/kg resulted in an almost complete loss of both adrenaline and noradrenaline, and recovery took several weeks.

In both experiments the concentrations of adrenaline and noradrenaline changed in parallel and in no instance was any absolute or relative increase above normal observed in the noradrenaline concentration. This is at variance with the results obtained by CALLINGHAM and MANN (1958a) and COUPLAND (1959). The former authors observed after 3 daily injections of 1mg/kg of reserpine first a decrease and after 3 days an increase in the noradrenaline content 300 % above the normal level. Adrenaline content also dropped first but it returned gradually to normal in the course of 2 weeks, during which time the temporarily elevated noradrenaline content slowly decreased to normal. In COUPLAND's (1959) experiment the noradrenaline content did not rise above the control level but it returned to normal before the adrenaline content; thus, the relative noradrenaline content temporarily increased. Moreover, COUPLAND (1959) made calculations suggesting that the noradrenaline content of those cells of the adrenal medulla which normally store mainly adrenaline was increased during the recovery phase.

Several factors must be considered in trying to explain the differences in the results obtained by CALLINGHAM and MANN (1958a), by COUPLAND (1959) and by us. First of all, the methods of catecholamine determination were different. CALLINGHAM and MANN (1958a) used rat's blood pressure and rat's uterus as bioassay techniques. These methods are subject to large errors when mixtures of adrenaline and noradrenaline are assayed (GADDUM and HOLZBAUER 1957). COUPLAND (1959) used a colorimetric method in which the differentiation of adrenaline and noradrenaline in a mixture is based on the fact that adrenaline is more readily oxidised at a low pH than noradrenaline. Also this method is subject to errors, particularly when much cortical extract, rich in ascorbic acid, and a relatively small amount of noradrenaline are present in the mixture, as is the case in the adrenal medulla of the rat. In our work the amines were first separated by paper chromatography, and thereafter an accurate fluorometric method of determination was used.

Fairly small errors in the estimation of the relative proportions of noradrenaline and adrenaline in COUPLAND's (1959) experiments would be sufficient to make non-significant the apparent small increase which he observed in the relative noradrenaline content during recovery. While COUPLAND's (1959) calculations seemed, furthermore, to suggest that there was an increase in the quantity of noradrenaline in the adrenaline-storing parts of the medulla, he did not histochemically observe any increase in the intensity of the iodate reaction in these areas. Even assuming that his figures for adrenaline and noradrenaline amounts were correct, the calculations which he made of the noradrenaline content of the medulla outside the specific cell islets are subject to criticism; they were based on measurements made by recording planimetrically the relative volume occupied by the noradrenaline-storing cell islets in the medulla, which he found decreased during the recovery.

Since the intensity of the iodate reaction was decreased and patchy in these cell islets during the recovery phase, COUPLAND (1959) himself found the delineation of the islets difficult and considered this in part responsible for the observed decrease in the volume of the islets after reserpine adminstration. Therefore, the apparently lower volume of the cell islets may indicate a temporary loss of noradrenaline from the noradrenaline-storing cells in the islets, rather than a real diminution in the size of the cell islets and an increase of noradrenaline concentration in the adrenaline-storing medullary cells outside the islets.

The observations made by COUPLAND (1959) and those of the present study are in agreement concerning the following points: reserpine caused in both studies a loss of both adrenaline and noradrenaline as well as a decrease in the intensities of the chromaffin and of the iodate reaction, and during the recovery phase there was no increase of the absolute noradrenaline content above the normal level. In both studies, furthermore, the intensity of the iodate reaction in the medullary background, *i. e.* in the cells normally storing adrenaline, did never increase above normal; indeed, our quantitative measurements indicated a decrease in this variable.

While COUPLAND's work and our differ, therefore, mainly in the interpretation, the results obtained by CALLINGHAM and MANN (1958a) are entirely different from those of COUPLAND and ours as far as changes in the noradrenaline content are concerned. It is difficult to find a pertinent explanation for this difference.

It does not seem likely that the very great increase which CALLINGHAM and MANN observed in the pressor response obtained with the extracts made from adrenals in the recovery phase could have been caused by errors in the assay. However, their evidence of the increase in the noradrenaline content is based on the assumption that the increase in the pressor response is due mainly to this amine. It is, however, not altogether impossible that the high assay values are in fact due to some other pressor substance whose concentration increases in the medulla during recovery.

Another possibility is that differences in the susceptibility of the rats towards reserpine in the different laboratories are of importance. Strain-dependent differences in the catecholamine loss after reserpine administration have earlier been demonstrated by COUPLAND (1958), who observed that Wistar rats required three times as much reserpine for an equal depletion as rats of the Sprague-Dawley strain. Dietary differences may also play a role in this respect.

Further studies are in progress on this problem in collaboration with Dr. K. R. BUTTERWORTH, who belongs to the same research group as Dr. MANN and Dr. CALLINGHAM.

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Distribution and Concentration of Adrenaline and Noradrenaline in the Adrenal Medulla of the Rat Following Depletion Induced by Muscular Work

By

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Abstract

ERÄNKÖ, O. and M. HÄRKÖNEN. *Distribution and concentration of adrenaline and noradrenaline in the adrenal medulla of the rat following depletion induced by muscular work.* Acta physiol. scand. 1961. *51.* 247—253. — The rats were allowed to run for 16 hours, to rest for 12 hours and to run again for 10 hours. They were killed immediately, 3, 6, 12 or 19 days thereafter. Concentrations of adrenaline and noradrenaline in the adrenal medulla were measured chemically. The intensities of the chromaffin reaction, the iodate reaction and formalin-induced fluorescence in adrenal sections were quantitated by microphotometry. Running caused an almost complete loss of both catecholamines from the adrenal medulla and the histochemical reactions turned almost negative. The catecholamine concentrations and the histochemical reactions returned to normal in 6 days. During the recovery phase the behaviour of the noradrenaline-storing medullary cells differed histochemically from that of the adrenaline-storing cells.

Muscular work is known to be a potent stimulator of the adrenal medulla (*e. g.* EULER 1956, KÄRKI 1956). However, we are not aware of studies in which the concentrations in the adrenal medulla of both adrenaline and noradrenaline had been studied after long-lasting muscular work. HÖKFELT (1951) reported that after swimming for 30—90 min the noradrenaline content of rat adrenals showed a tendency to rise, whereas the adrenaline content

tended to decline. He did not study the effect of longer-lasting work. Since interesting features in the recovery of the catecholamines have been reported after depletion by pharmacological substances, *e. g.* reserpine (CALLINGHAM and MANN 1958 a, b, COUPLAND 1959, ERÄNKÖ and HOPSU 1961), we undertook a similar study using muscular work as a stimulus.

Material and Methods

Experimental

Adult albino rats were used. They were allowed to run in a rotating wire cylinder for varying periods. In preliminary experiments the effect on the adrenal medulla was investigated histochemical methods only, using the chromaffin reaction, the iodate reaction and formalin-induced fluorescence (see below) as indicators of the amount of the catecholamines in the medulla. In these experiments, a single period of work lasting up to several hours resulted only in a partial depletion of the medullary catecholamines, and a rapid restoration to normal occurred within a day after the work had been stopped.

However, when the rats were allowed to run for 16 hours, to rest thereafter for 12 hours and to run again for 10 hours, an almost total disappearance of catecholamines was observed, as indicated by the three histochemical reactions, which all turned almost negative. This experimental arrangement, which proved reproducible, was then adopted as a routine procedure, and the results to be reported in this paper have been obtained by using it.

The animals were killed by decapitation inmediately, 3, 6, 12 or 19 days after the end of the second running period, together with about the same number of similar but untreated control rats. Both adrenals were cut into two pieces of equal size. One piece was used for chemical analysis, the remaining 3 pieces for histochemical studies.

Chemical methods

Fresh adrenal sections cut at 50 μ were freeze-dried and pieces of pure dry medulla were dissected out and weighed. The catecholamines were either first separated by chromatography, eluted and determined separately by fluorometry after oxidation at pH 6 (*e. g.* EULER and FLODING 1955) or determined directly in the extract, making the fluorometric measurements at two exciting wavelengths, 366 mμ and 436 mμ for the determination of the relative proportion of noradrenaline in the mixture (*e. g.* COHEN and GOLDENBERG 1957). For further details of the method, see ERÄNKÖ and RÄISÄNEN (1960). Since the two methods gave comparable results, the data obtained with them were pooled and handled together.

Histochemical methods

One of the three remaining adrenal pieces was immersed in a mixture of potassium dichromate and formalin for the demonstration of both catecholamines by the chromaffin reaction, another was plunged into a saturated solution of potassium iodate for the demonstration of noradrenaline (HILLARP and HÖKFELT 1955), and the third one was fixed in a mixture of calcium chloride and formalin to develop the formalin-dependent fluorescence in the noradrenaline cell islets. Frozen sections cut at 50 μ were mounted in glycerol. For details see the previous paper (ERÄNKÖ and HOPSU 1961).

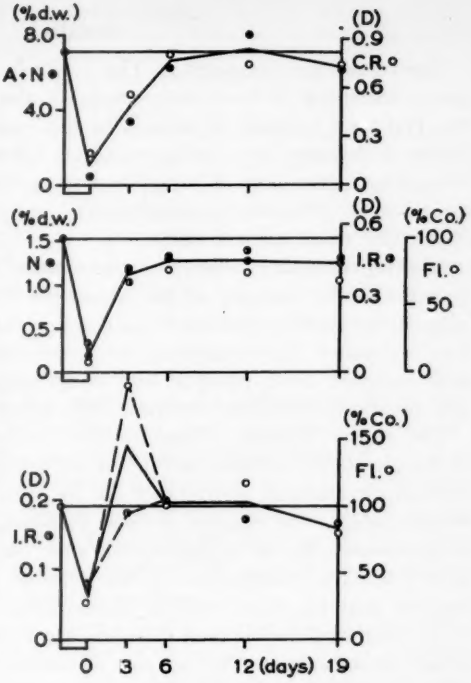

Fig. 1. Effect of muscular work on catecholamines of the adrenal medulla.

The running period is indicated by a white rectangle below the base line. Each circle represents the mean of a group of 5—7 animals. The solid line has been drawn between the mean chemical and histochemical values.

Top graph: Concentration of both catecholamines (A + N; full circles), % of dry medullary weight, and optical density (D) of the chromaffin reaction (C. R.; open circles).

Center graph: Concentration of noradrenaline (N; full circles), % of dry medullary weight; optical density (D) of the iodate reaction (I. R., half-filled circles) and intensity of the formalin-induced fluorescence (Fl.; open circles), % of the corresponding mean control value. The intensities of the iodate reaction and the fluorescence were measured from the noradrenaline-storing cell islets.

Bottom graph: Iodate reaction and formalin-induced fluorescence in the medullary "background", *i.e.* adrenaline-storing cells. Note that fluorescence is expressed as % of the corresponding (*i. e.* background) mean control value. Therefore the absolute values of fluorescence in this and the center graph are not comparable.

Quantitative measurement of histochemical reactions

The optical densities of the chromaffin reaction and the iodate reaction, as well as the intensity of the formalin-induced fluorescence were measured by microphotometry. The method has been described in detail elsewhere (ERÄNKÖ and RÄISÄNEN 1960, see also ERÄNKÖ and HOPSU 1961). Measurements of the intensities of the iodate reaction and the formalin-induced fluorescence were carried out separately on the noradrenaline-containing cell islets and on the medullary background consisting of cells normally storing mainly adrenaline.

Measurement of medullary volume

A separate experiment was carried out to study the effect of running on the volume of the adrenal medulla. The animals were killed immediately, 3 or 6 days after running in two consecutive days according to the schedule described earlier in this paper. One adrenal was immersed in the dichromate-formalin mixture, the other in a saturated iodate solution. After 24 hours, both glands were postfixed in 4 % formaldehyde. The medullary volume was planimetrically measured from a complete series of sections cut from the gland fixed in dichromate-formalin (ERÄNKÖ 1954).

Results

The results are presented in Fig. 1. Each circle represents a mean of a group consisting of 5—7 rats, excepting the controls, whose number was 28. The total number of animals in this experiment was 58. The running period is indicated by a white rectangle below the base line. The scales of each graph have been adjusted by setting the mean control values at the same height. The fluorescence intensity is expressed as percent of the corresponding mean control value.

The top diagram indicates a pronounced loss of catecholamines and a decrease in the intensity of the chromaffin reaction immediately after running. Both variables returned to normal in 6 days, and remained at the normal level thereafter. The changes in the catecholamine content and in the chromaffin reaction were parallel, and in no stage of the experiment was there any significant difference between their mean values.

The center diagram illustrates the changes in the noradrenaline concentration of the medulla and in the intensities of the iodate reaction and of the formalin-induced fluorescence in the specific cell islets normally storing noradrenaline. The changes in these variables are closely associated showing a pronounced loss of noradrenaline after running and a return to normal in a few days. Comparison of this diagram and the upper one seemingly suggests that the noradrenaline concentration was nearer the normal value three days after the running than was the total catecholamine concentration, which would indicate that the rate of formation of noradrenaline was higher than that of adrenaline. However, there was no statistically significant difference on the third day between the mean of the total catecholamine content expressed as percent of the control mean and that of the mean noradrenaline content expressed in the same way. Although the means of the three variables in the center diagram remained below the corresponding normal mean on the 6th, 12th and 19th day, the differences from the corresponding control means were not statistically significant.

The bottom diagram illustrates changes in the intensities of the iodate reaction and of the formalin-induced fluorescence in the medullary "background", *i. e.*, in the cells normally storing adrenaline. Because the scales have been adjusted by setting the mean control value, which is much smaller than in the cell islets, at the same height as in the two upper graphs, smaller absolute changes correspond to relative changes equal to those in the upper graphs.

Running caused a decrease in the intensities of the iodate reaction and the formalin-induced fluorescence in the background, and the values returned quickly towards normal. On the third day, the intensity of the iodate reaction was already at the control level. This is of interest because neither the noradrenaline concentration in the medulla nor the histochemical reactions in the noradrenaline cell islets had returned to normal during the same time.

Table I. Effect of running on the volume of the adrenal medulla

Treatment	Number of animals	Volume of medulla (per cent of controls)		P[1]
		Mean	SD	
Controls	8	100.0	9.6	—
Running, killed immediately....	9	126.4	19.1	~ 0.003
Running, killed after 3 days....	7	108.5	6.3	> 0.05

[1] The value of P has been calculated with the aid of the t-test and shows the significance of the difference from the control group.

The difference of the means of the intensity of the iodate reaction in the specific cell islets and that in the background, both expressed in terms of percent of the corresponding control mean, is statistically significant ($P \sim 0.02$).

The most striking feature in this graph is, however, the very high fluorescence intensity of the background on the 3rd day. This increase is statistically highly significant ($P < 0.001$). From the 6th day on the intensity of both the iodate reaction and the fluorescence kept near the corresponding control mean and did not differ from it significantly.

Table I shows the changes in the volume of the adrenal medulla immediately and 3 days after the last running period. Running caused a 26 percent increase in the medullary volume but after 3 days the mean volume did not any more differ significantly from that of the control group. The chromaffin reaction and the iodate reaction were almost negative immediately after running and their intensities were according to visual judgement about normal after 3 days' rest. However, after 3 days the intensity of the iodate reaction in the background of the medulla tended to be higher than in the controls.

Discussion

Long-lasting muscular work caused in the present study a pronounced depletion of both adrenaline and noradrenaline from the adrenal medulla. It is not possible to say in how far this depletion was due to muscular work alone and how much other factors such as general exhaustion, lack of sleep and diminished intake of food and water contributed to the effect observed. Fasting or lack of water for several days cause hardly any changes in the intensity of the chromaffin reaction in the adrenal medulla of the rat (ERÄNKÖ, unpublished) but they both may nevertheless potentiate the effect of muscular work. However, whatever the mechanism of the depletion is, it was reproducible and reversible.

The concentrations of both catecholamines and the intensities of the histochemical reactions employed returned to normal in 6 days. This period is much shorter than that required for recovery after reserpine administration, which resulted in a comparable initial loss of catecholamines from the adrenal medulla (*cf.* Fig. 1 in this paper with Fig. 1 in ERÄNKÖ and HOPSU 1961). Such a difference suggests that reserpine seriously interferes with the synthesis and/or storage of adrenaline and noradrenaline in the adrenal medulla, while even prolonged muscular work has less or no such effect. This fits well in with the recent observations demonstrating that reserpine prevents the storage of catecholamines in the cytoplasmic granules (BERTLER, HILLARP and ROSENGREN 1960).

In general, the results of the chemical determinations agreed in the present study well with those obtained by quantitating the histochemical reactions. Likewise, the changes in the adrenaline and noradrenaline concentrations were parallel. In these respects the results were similar as those obtained in the reserpine experiment (ERÄNKÖ and HOPSU 1961).

However, 3 days after the end of the running period, *i. e.* when an active resynthesis of the depleted amines took place, an interesting divergence was observed between the intensities of the iodate reaction and the formalin-induced fluorescence in the medullary background: while the intensity of the iodate reaction was equal to that in normal controls, the intensity of the fluorescence was almost twice the normal value.

This shows that the formalin-induced fluorescence, although it usually is parallel with the iodate reaction, is not always so. For this there are three possible explanations. First, the formalin-induced fluorescence may not, like the iodate reaction, depend, at least exclusively, on noradrenaline but to some other substance whose concentration tends usually but not always to vary parallelly with that of noradrenaline. Second, the increase in the intensity of the fluorescence is perhaps not due to an increase in the amount of the fluorescent substance but may be caused by some other change, *e. g.* by a loss of a substance quenching the fluorescence; this explanation is compatible with the assumption that noradrenaline is responsible for the fluorescence. Third, the iodate reaction may not demonstrate noradrenaline outside the granules.

Of these alternatives the first one appears most likely but the nature of the postulated fluorescent substance, if other than noradrenaline, remains obscure. At any rate, the observation completes two earlier ones in which a discrepancy was observed between the intensities of the fluorescence and the iodate reaction (ERÄNKÖ 1955, ERÄNKÖ, HOPSU and RÄISÄNEN 1959).

On the 3rd day, the intensity of the iodate reaction in the medullary background was already normal although the total catecholamine concentration and the noradrenaline concentration were still below normal. Assuming that the iodate reaction is a faithful indicator of the local noradrenaline concentra-

tion, this means that the rate of formation of noradrenaline in the normally adrenaline-storing cells was higher than the rate of the subsequent methylation to noradrenaline. It is noteworthy that CALLINGHAM and MANN (1958 a, b) and COUPLAND (1959) arrived at the same conclusion in experiments in which the loss of catecholamines was induced by reserpine. However, since the results obtained in this laboratory with reserpine (ERÄNKÖ and HOPSU 1961) are different and since the matter is under investigation in collaboration with the London laboratory, it seems better to postpone the discussion of this matter until the results of the joint study are available.

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The Effect of Testosterone, Progesterone and Estrogen on the Acetylating Activity in Rat Liver and Kidney Preparations

By

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Abstract

LUUKKAINEN, T. *The effect of testosterone, progesterone and estrogen on the acetylating activity in rat liver and kidney.* Acta physiol. scand. 1961. *51.* 254—262. — The acetylation of *p*-aminobenzoic acid has been studied *in vitro* in rat liver and kidney preparations after the treatment of the rats with gonadal steroids. It was observed that none of these steroids in the doses used had any effect on the acetylation in the liver tissue. In the kidney tissue of male rats estrogen treatment clearly decreased the acetylation, progesterone had no effect, and testosterone may increase the acetylating kidney tissue in female rats.

The capacity of male and female rats to acetylate aromatic amines has been found to be different. Reports of DUMM and RALLI (1951), FRANZ and LATA (1953, 1957) and FISHKIN and LATA (1957, 1958) have indicated that male rats *in vivo* have a greater acetylating activity than female rats. It has also been demonstrated that sex hormones administered to rats are capable of changing their acetylation power *in vivo* (FISHKIN and LATA 1958).

The sex-linked difference in the acetylation was found in this laboratory to exist also *in vitro*, where some of the disturbing factors of the *in vivo* experiments can be eliminated (LUUKKAINEN 1958). The effect of sex glands on acetylation was clearest in the kidney tissue. The kidney preparations of the male rats acetylated larger amounts of *p*-aminobenzoic acid than those of the female rats. Castration of the male rats reduced the acetylating activity, whereas ovariectomy increased the acetylation in the kidney preparations.

This communication deals with the effects of gonadal steroid treatment on the *in vitro* acetylation of the rat liver and kidney tissue.

Materials and Methods

The rats used were of the Wistar strain. The rats for the experiments were selected by weight from healthy animals of the same age. The animals were first tested *in vivo* with *p*-aminobenzoic acid (PAB) to find out their acetylating power. In the *in vivo* tests the rats were given 15 μmoles of PAB through a stomach tube in 5 ml of water. Urine was then collected during an experimental period of 4 hours and the urine samples were analyzed to determine the acetylation percentage and amount of amine excreted. On the basis of these tests the males with the highest and the females with the lowest acetylation power were castrated to make the biological variation between the experimental animals as small as possible.

One month later the animals were tested again *in vivo* with PAB. According to the results of this test each of the four groups of animals, *i. e.*, the unoperated as well as the castrated male and female rats, were divided into control and experimental groups having a nearly equal acetylation ability.

The steroid preparations used were as follows:

Testosterone — Testosterone propionate and testosterone enanthate (Primoteston and Primoteston depot, Schering AG, Berlin).

Progesterone — Progesterone and 17 α-hydroxyprogesterone caproate (Primolut and Primolut depot, Schering AG, Berlin).

Estrogen — Estradiol benzoate (Primogyn B oleosum, Schering AG, Berlin).[1]

The hormones were diluted with their original solvent oils to the desired concentrations and given intramuscularly to the experimental rats in 0.1 ml portions. The control animals were treated with the same volume of the corresponding solvent oil.

The effect of the treatment was followed by subjecting the rats to the *in vivo* acetylation tests.

For the *in vitro* experiments the animals were fasted for one night and killed by decapitation. The viscera were removed and quickly placed on ice. From each rat two 2 g liver samples and both kidneys were weighed and used for the experiments. The tissues were dropped into incubation flasks containing 10 ml of ice cold incubation medium of the following composition:

Physiological sodium chloride solution	200 ml
0.154 M potassium chloride solution	8 ml
1.0 M sodium acetate solution	8 ml
0.02 M neutralized *p*-aminobenzoic acid	4 ml
0.1 M potassium sodium phosphate buffer, pH 7.4 to make	400 ml

The volume of medium used thus contained 2 μmoles of PAB and 200 μmoles of acetate for 2 g of liver tissue or for one kidney.

The tissue was minced in the incubation flasks with scissors into small sections as described previously (LUUKKAINEN 1958). Then the flasks were gassed for 10 minutes with oxygen and after this procedure incubated in a water-bath at 37° C with constant shaking. After incubation, duplicate samples of 1 ml were taken from each flask and transferred to centrifuge tubes containing 4 ml of 5 per cent trichloroacetic acid for deproteinization. The determination of the *p*-aminobenzoic acid conjugates was made by the method of BRATTON and MARSHALL (1939), calculating the amount of acetylated conjugates from values obtained without hydrolysis and after hydrolysis.

[1] I wish to express my gratitude to Messrs. Schering AG for kindly supplying the steroids.

Results

There was no observable difference between rats treated with testosterone and control rats when they were tested *in vivo* with *p*-aminobenzoic acid twice a month during the treatment. The dose of testosterone used was 500 μg of testosterone propionate i. m. per day for 28 days, then 3.5 mg of testosterone enanthate i. m. weekly for the following 6 weeks, and testosterone propionate 500 μg per day i. m. for the remaining 20 days. The dose was thus 500 μg per day during 90 days. This dose had no effect on the acetylating capacity of liver *in vitro*, as shown in Table I.

Table I. Acetylation of p-aminobenzoic acid (PAB) in liver after testosterone treatment

Each incubation flask contained 2 g of liver sections, 2 μmoles of PAB and 200 μmoles of acetate in 10 ml of medium, pH 7.4. Oxygen as gas phase and incubation time 4 hours. Testosterone 500 μg i. m. per day for 90 days before experiment.

Sex of rats	Treatment	Number of rats	Acetyl-PAB formed, μmoles	S. D.	S. E.	Significance of change from controls
Females	Testosterone	5	0.72	0.015	0.007	None
	Control	4	0.77	0.147	0.073	($P > 0.05$)
Castrated females	Testosterone	6	0.62	0.094	0.042	None
	Control	5	0.68	0.093	0.041	
Males	Testosterone	4	0.67	0.131	0.066	None
	Control	5	0.73	0.068	0.031	
Castrated males	Testosterone	6	0.72	0.067	0.027	None
	Control	5	0.71	0.060	0.027	

In the kidney, as seen from Table II, the amount of acetylated *p*-aminobenzoic acid formed per gram of kidney tissue is smaller in testosterone treated castrated male rats than in corresponding control rats. However, there is the typical testosterone effect (KORENCHEVSKY and DENNISON 1934, KORENCHEVSKY *et al.* 1937 (a) and 1937 (b), *i. e.* increasing weights of kidneys, as stated in Table III. This effect naturally influences only the results of the treated animals. For comparison with the *in vivo* experiments reported in the literature, Table II also shows the acetylated conjugates formed per whole kidney. As in the *in vivo* experiments the change in weight of the kidneys then remains unaccounted for. The kidneys of ovariectomized treated female rats and perhaps of unoperated treated female rats produce more acetylated conjugates than the corresponding controls when this manner of calculation is used. The total acetylating capacity of the kidney is the same in the treated and control groups of male and castrated male rats.

Table II. Acetylation of p-aminobenzoic acid (PAB) in kidney after testosterone treatment

One kidney as tissue sample other experimental conditions as in Table I.

Sex of rats	Treatment	Acetyl-PAB formed per 1 g of kidney μmoles	S. D.	S. E.	Significance	Acetyl-PAB formed per kidney, μmoles	Significance of change from controls
Females	Testosterone	0.57	0.089	0.040	None	0.55	+, $P < 0.02$
	Control	0.45	0.072	0.036		0.33	
Castrated females	Testosterone	0.64	0.075	0.031	None	0.69	+, $P < 0.01$
	Control	0.55	0.091	0.040		0.50	
Males	Testosterone	0.65	0.131	0.066	None	0.71	None
	Control	0.90	0.068	0.031		0.91	
Castrated males	Testosterone	0.66	0.095	0.039	$-P<0.05$	0.80	None
	Control	0.82	0.051	0.023		0.73	

Table III. Body and organ weights of testosterone treated rats

Sex of rats	Treatment	Mean of body weights, g	Mean of liver weights, g	Mean of kidney weights, g
Females	Testosterone	187	7.490	0.960
	Control	170	7.560	0.740
Castrated females	Testosterone	230	8.760	1.080
	Control	245	9.250	0.910
Males	Testosterone	214	8.270	1.090
	Control	268	9.680	1.010
Castrated males	Testosterone	251	9.590	1.220
	Control	238	8.610	0.890

The dose of progesterone was 500 μg per day i. m. for 28 days, followed by 6 weeks' administration of 3.5 mg i. m. hydroxyprogesterone caproate weekly and after this again 500 μg of progesterone per day for 20 days.

The results of the experiments with liver tissue are presented in Table IV. There are no statistically significant differences in the acetylation between treated and control rats. The average amount of formed acetylated *p*-aminobenzoic acid conjugates in the progesterone treated male rats is slightly lower than in the untreated rats, probably reflecting only slight "hormone castration" caused by progesterone.

Table IV. Acetylation of p-aminobenzoic acid (PAB) in liver after progesterone treatment

Each incubation flask contained 2 g of liver sections, 2 μmoles of PAB and 200 μmoles of acetate in 10 ml of medium, pH 7.4. Oxygen as gas phase and incubation time 4 hours. Progesterone 500 μg i. m. per day for 90 days before experiment.

Sex of rats	Treatment	Number of rats	Acetyl-PAB formed, μmoles	S. D.	S. E.	Significance of change from controls
Females	Progesterone	10	0.60	0.09	0.028	None
	Control	9	0.65	0.09	0.030	($P > 0.05$)
Castrated females	Progesterone	9	0.65	0.38	0.126	None
	Control	9	0.64	0.38	0.126	
Males	Progesterone	10	0.70	0.44	0.140	None
	Control	9	0.87	0.09	0.030	
Castrated males	Progesterone	8	0.63	0.47	0.170	None
	Control	9	0.59	0.07	0.025	

On examination of the mean values of the acetylated conjugates formed in the kidney preparations of progesterone treated and control rats it is observed that there are no significant differences between these two groups (Table V). Table VI shows the mean weight of body, liver and kidney of rats in the progesterone series. There is no kidney enlargement, as after testosterone treatment (Table III).

Table V. Acetylation of p-aminobenzoic acid (PAB) in kidney after progesterone treatment

Each incubation flask contained one kidney in sections, other experimental conditions as in Table IV.

Sex of rats	Treatment	Acetyl-PAB formed per 1 g of kidney, μmoles	S. D.	S. E.	Significance of change from controls
Females	Progesterone	0.36	0.01	0.003	None
	Control	0.36	0.05	0.016	($P > 0.05$)
Castrated females	Progesterone	0.40	0.06	0.019	None
	Control	0.49	0.08	0.025	
Males	Progesterone	0.72	0.36	0.113	None
	Control	0.79	0.06	0.020	
Castrated males	Progesterone	0.56	0.06	0.022	None
	Control	0.59	0.08	0.027	

Table VI. Body and organ weights of progesterone treated rats

Sex of rats	Treatment	Mean of body weights, g	Mean of liver weights, g	Mean of kidney weights, g
Females	Progesterone	179	7.720	0.760
	Control	169	7.150	0.760
Castrated females	Progesterone	217	9.320	0.890
	Control	225	9.130	0.890
Males	Progesterone	281	11.060	1.060
	Control	297	11.210	1.160
Castrated males	Progesterone	262	10.730	0.990
	Control	285	9.580	1.020

When the effect of estrogen was studied, estradiol benzoate was given 2 μg per day i. m. for 120 days. The duration of the treatment was so long because in the *in vivo* tests there were some rather insignificant decreases in the acetylation of male rats after 90 days. This decrease did not come out more clearly by waiting the following 30 days. The results of the experiments with liver tissue are presented in Table VII. We see that there are no differences in the acetylation after the estrogen treatment between the treated and control rats.

Table VII. Acetylation of p-aminobenzoic acid (PAB) in liver after estrogen treatment

Each incubation flask contained 2 g of liver sections, 2 μmoles of PAB and 200 μmoles of acetate in 10 ml of medium, pH 7.4. Oxygen as gas phase and incubation time 4 hours. Estradiol benzoate 2 μg i. m. per day for 90 days before experiment.

Sex of rats	Treatment	Number of rats	Acetyl-PAB formed, μmoles	S. D.	S. E.	Significance of change from controls
Females	Estradiol	5	0.79	0.11	0.049	None
	Control	5	0.77	0.06	0.029	($P > 0.05$)
Castrated females	Estradiol	6	0.80	0.07	0.027	None
	Control	5	0.81	0.16	0.071	
Males	Estradiol	5	0.71	0.14	0.061	None
	Control	5	0.74	0.11	0.048	
Castrated males	Estradiol	7	0.70	0.10	0.036	None
	Control	5	0.70	0.06	0.025	

The same small dose of estradiol, which in the liver has no effect, clearly decreases the acetylating ability of the kidney. This decreasing effect of estrogen comes out already in the examination of the average amounts of acetylated conjugates in μmoles (Table VIII). The numerical values of formed acetyl-PAB are smaller in all the treated animals than in the controls. The effect is, however, statistically significant only in both male rat groups. As seen from Table IX, this decrease is not an indication of change in the weight of the kidneys.

Table VIII. Acetylation of p-aminobenzoic acid (PAB) in kidney after estrogen treatment

Each incubation flask contained one kidney in sections, other experimental conditions as in Table VII.

Sex of rats	Treatment	Acetyl-PAB formed per 1 g of kidney, μmoles	S. D.	S. E.	Significance of change from controls
Females	Estradiol	0.25	0.01	0.007	None
	Control	0.31	0.07	0.029	
Castrated females	Estradiol	0.31	0.07	0.029	None
	Control	0.42	0.01	0.006	
Males	Estradiol	0.31	0.02	0.007	$-$, $P < 0.01$
	Control	0.57	0.07	0.032	
Castrated males	Estradiol	0.30	0.01	0.003	$-$, $P < 0.001$
	Control	0.58	0.06	0.025	

Table IX. Body and organ weights of estrogen treated rats

Sex of rats	Treatment	Mean of body weights, g	Mean of liver weights, g	Mean of kidney weights, g
Females	Estradiol	197	8.100	0.930
	Control	215	8.390	0.930
Castrated females	Estradiol	202	9.320	0.920
	Control	237	8.080	0.880
Males	Estradiol	248	9.370	0.940
	Control	271	10.060	1.060
Castrated males	Estradiol	249	10.040	0.980
	Control	263	8.890	0.880

Discussion

The ability of female rats to acetylate arylamine has been shown by FISHKIN and LATA (1957, 1958) to be increased after testosterone treatment *in vivo*. In the present investigation no differences were found between the experimental and control animals in the acetylation of liver tissue after testosterone treatment. Testosterone increased the weight of the kidneys, as shown earlier by KORENCHEVSKY and DENNISON (1934) and KORENCHEVSKY *et al.* (1937 a, 1937 b). This increase in weight is an indication that the dose of steroid used has had an effect. However, there is no effect on the acetylation when calculated per gram of kidney. It has to be borne in mind, however, that the total acetylating capacity of the kidneys increases in the treated female and ovariectomized groups. This finding corresponds to the above mentioned *in vivo* results. *In vivo* the effects of the kidney enlargement cannot be observed.

In the progesterone group there is no difference between treated and untreated rats in the acetylation in either tissues tested. This observation supports the idea that progesterone alone has no influence on the sex-linked difference found in arylamine acetylation.

Estrogen treatment has, according to the presented results, no effect on liver acetylation. Estrogen acts in a different manner in the kidney, where it is a potent depressor of acetylation, even at a relatively low daily dose (2 μg) of estradiol. The inhibition of acetylation is in agreement with previous findings, where ovariectomy elevated the level of the acetylation (LUUKKAINEN 1958). The observed decrease in acetylation does not seem to be due to activation of a deacetylating enzyme in the used tissue preparations, where no deacetylation of the acetylated *p*-aminobenzoic acid was found (KOIVUSALO and LUUKKAINEN 1959).

The different sensitivity of acetylation to estrogen in the kidney and liver are presumably based on a difference in the conjugation system in these two organs and in the kidney it may have some correlation with the sex-dependent differences in structure. Morphological structure differences have been found to exist in the kidney between the two sexes (CRABTREE 1940, ESCHENBRENNER and MILLER 1945). The role of the kidney in the acetylation difference between male and female rats, also *in vivo* might be of some importance.

I wish to express my thanks to the Sigrid Jusélius Foundation for a grant which enabled me to carry out this work.

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The Urinary Excretion of Galactose and its Significance in Clinical Intravenous Galactose Tolerance Tests

By

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Abstract

TYGSTRUP, N. *The urinary excretion of galactose and its significance in clinical intravenous galactose tolerance tests.* Acta physiol. scand. 1961. *51.* 263—274. — The disappearance of galactose from the blood after intravenous administration may be used for measurement of the liver function, if correction for the urinary loss of galactose is made. The present study was performed in order to determine the significance of this loss. It was found that urinary excretion of galactose was unimportant in relation to hepatic elimination at plasma concentrations below about 500 mg/l (approximately 3 per cent of the total elimination), but at higher concentrations the ratio between urinary excretion and hepatic elimination increased, as the former rises with the concentration, and the latter remains constant. After a single injection of about 500 mg/kg body weight on an average 10 per cent of this amount was excreted in normal subjects as well as in patients with cirrhosis. This percentage could be used for a practical calculation of the contribution of the urinary loss to the total elimination rate of galactose during the interval in which the latter remains essentially constant. The experiments indicate a low renal threshold for galactose, but no evidence for a reabsorption T_m was found within the range of plasma concentrations studied (up to 3,000 mg/l). The renal clearance of galactose was significantly decreased in patients with cirrhosis. The implications of these findings for the calculation of the maximal hepatic galactose elimination rate from the plasma concentration curve following a single injection are examined in an appendix.

The fact that the plasma galactose concentration-time curve following a single intravenous injection of galactose is essentially rectilinear over a considerable range of plasma concentrations (TYGSTRUP and WINKLER 1954), indicates that in this range the elimination of galactose mainly depends on processes which are saturated at such concentrations. As galactose is predominantly eliminated by the liver, the maximal galactose elimination rate is determined largely by the metabolic status of the liver.

It is well known, however, that some extrahepatic elimination of galactose does occur (BOLLMAN, MANN and POWER 1935), chiefly by way of renal excretion. If urinary loss of galactose is significant under the conditions used in clinical intravenous galactose tolerance tests, determination of the hepatic galactose elimination rate from elimination curves in plasma requires correction for this extrahepatic elimination.

The renal excretion of galactose in patients with normal and with reduced liver function therefore has been investigated in order to determine the magnitude of this correction. The renal excretion was studied partly under conditions of continuous intravenous galactose infusions (with constant or constantly rising plasma galactose concentrations) and partly under conditions of falling plasma concentrations (following a single intravenous injection).

Methods

The patients were studied in the morning while lying in their bed, more than 12 hours after their last meal. They were allowed to drink moderate amounts of water, but forced drinking to ensure high diuresis was avoided, as this apparently influences the excretion of galactose.[1]

Galactose was administered as analyzed solutions by calibrated, motor-driven syringes. Infusions of galactose were maintained for one to three hours, and the urine samples examined were collected either in periods with constant plasma concentrations, or in periods with constantly rising concentrations, depending on whether the galactose infusion rate was smaller or greater than the maximal galactose elimination rate of the patient.

In experiments with single injection of galactose about 500 mg/kg body weight was injected at a constant rate in the course of 6 minutes.

Determination of galactose in plasma was performed as previously described (TYGSTRUP *et al.* 1954). As galactose is mainly distributed in extracellular fluid (except in the liver), concentrations are expressed as mg/l plasma water.

Urine was collected through an indwelling bladder catheter. The bladder was emptied by suction, washed twice with 20 ml of sterile saline, and urine and washing fluid were mixed.

When the excretion was studied at falling or rising plasma concentrations, the galactose of the urine samples was related to the mid-concentration in plasma 8 minutes

[1] In 2 patients a single injection of the same amount of galactose was given during high and low diuresis. After deprivation of water for 24 hours the urine flow of these patients during the test was 0.55 and 0.60 ml/min, and after administration of one liter of water in the morning 8.0 and 6.6 ml/min. The excretion of galactose was increased by 29 and 36 per cent, respectively, during high diuresis.

prior to the mid-time of the urine collection interval. This correction for the average delay-time in the urinary tract was applied because in one experiment (see Fig. 2) in which the excretions were studied during rising as well as during falling plasma concentrations, application of this correction resulted in essentially identical renal clearances at comparable plasma concentrations.

For determination of galactose in urine the specimen was diluted to an estimated concentration of about 500 mg/l. The reducing power was determined by the same method as used for plasma, partly after treatment with a galactose fermenting strain of yeast,[1] partly without treatment. The yeast, cultured in a galactose containing broth, was washed three times in saline to remove reducing substances. About 5 g of washed yeast was suspended in 30 ml of 0.2 M phosphate buffer (pH = 5.6). Two ml of diluted urine and 2 ml of yeast suspension were mixed in a flask which was stoppered, placed in a water bath at + 30 C, and automatically shaken for 90 min.

The difference between reduction values of the yeast-treated and the untreated sample was taken as concentration of galactose. Treatment with yeast for determination of the unfermentable (non-galactose, non-glucose) rest reduction was found necessary, especially when the concentration of galactose was low, as the rest reduction varied widely from sample to sample, and in most cases amounted to 500—1,500 mg/l of urine, expressed as galactose. The standard deviation of this analytical procedure is about 3 per cent.

As glucose is fermented by this strain of yeast as well as galactose, glucosuria will give falsely high values. In earlier experiments, when glucosuria was suspected from previous urinalysis, galactose was determined as the difference between a yeast-treated and a Notatin[2] treated sample. In more recent experiments this procedure was used when the urine showed reaction with Notatin-impregnated paper (Clinistix®).

Results

A. *Infusion experiments with constant plasma concentration*

The rate of galactose infusion was lower than the maximal galactose elimination rate of the patients. In patients with normal liver function the infusion rate was about 300 mg/min, and in patients with diseases of the liver about 200 mg/min. Consequently the plasma concentrations were lower than 400 to 500 mg/l, *i. e.* the level at which the eliminating mechanism becomes saturated (Tygstrup and Winkler 1954). The material comprises 32 determinations in 29 subjects with normal hepatic and renal function and 10 determinations in patients with cirrhosis of the liver or acute epidemic hepatitis.

In the subjects with normal liver function the rate of galactose excretion averaged 9 mg/min (s. d. 5.6) or 3.1 per cent of the infusion rate, and in the patients with reduced elimination of galactose 4.1 mg/min (s. d. 3.1) or 1.8 per cent of the infusion rate. Fig. 1 shows the relationship between the amount of galactose excreted in the urine per minute and the concentration in plasma water. It is seen that the correlation between the two is poor, and the calculated renal clearance of galactose therefore varies widely. In patients with normal liver function it averages 35 ml/min (s. d. 18), and in the patients with hepatic diseases 17 ml/min.

[1] Kindly supplied by Carlsberg Laboratories, Copenhagen.

[2] Generously supplied by Leo Pharmaceutical Ltd., Copenhagen.

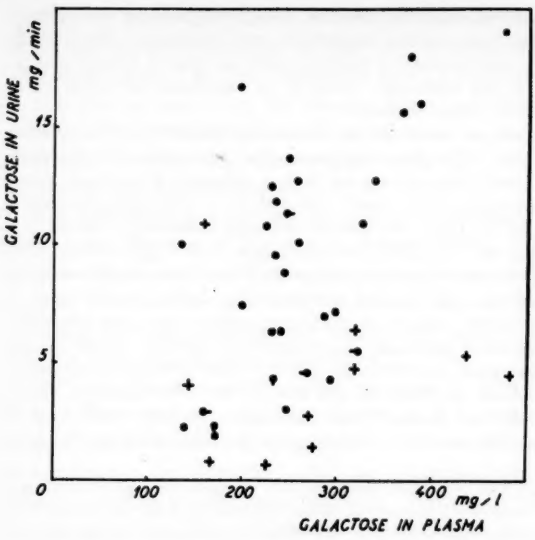

Fig. 1. Urinary excretion rate of galactose at low, constant plasma concentrations.

• = subjects with normal liver function,

+ = patients with diseases of the liver.

•–, ⁄•, and –• are patients in whom two successive determinations were performed.

B. *Infusion experiments with rising plasma concentration*

The rate of galactose infusion was between 400 and 800 mg/min which was greater than the maximal galactose elimination rate of the patients. The material consists of 8 subjects with normal liver function and 3 patients with cirrhosis of the liver. Most data were obtained in intervals during which the concentrations were rising at rates varying from 0.2 to 12 mg/l/min. In one case the urinary excretion during a steeper rise (34 mg/l/min) was compared to that occurring during a falling curve (— 51 mg/l/min) found after interruption of the infusion. (This experiment was used for assessment of the average delay-time in the urinary tract.)

The relationship between the urinary excretion rate of galactose and the concentration in plasma water is shown in Fig. 2. In this concentration interval the correlation is more definite than at low concentrations, although the individual variation is still appreciable. Furthermore it is seen that the renal clearance tends to fall at low concentrations. In the intermediate concentration interval of 1,000 to 2,000 mg/l it averages 46 ml/min in the subjects with normal liver function, and at higher concentrations 57 ml/min.

C. *Single injection experiments*

The rate of galactose elimination may be calculated from the rectilinear plasma concentration-time curve by multiplying the absolute value of the slope of the curve by the volume of distribution of galactose, the latter being

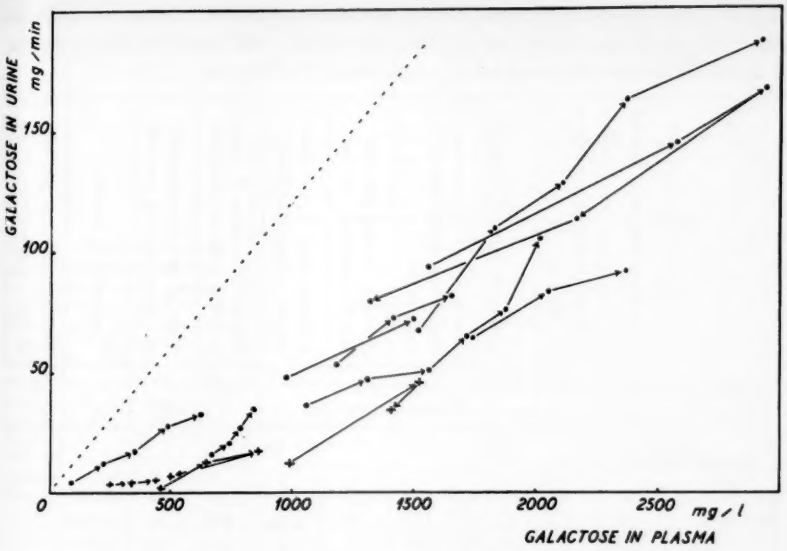

Fig. 2. Urinary excretion rate of galactose at higher, changing plasma concentrations. The arrows indicate whether the observation was made during rising or falling concentration. • = subiects with normal liver function, + = patients with cirrhosis of the liver. The dotted line indicates the amount of galactose filtered per minute at different plasma concentrations, assuming the glomerular filtration rate to be 120 ml/min.

obtained by dividing the amount injected by the extrapolated concentration at zero time. The rectilinear part of the curve, the *test period*, generally starts about 20 minutes after beginning of the injection and ends when the concentration falls below about 400 mg/l.

Quantitative collection of the urine excreted during the entire test period is not practicable, as the exact duration of this period is not known beforehand in the individual patient. In about one fourth of the experiments the test period was found to be inadequately covered by the urine collection intervals employed. Excluding these, 9 experiments remained in subjects with normal liver function, and 32 in patients with cirrhosis of the liver which were satisfactory for an evaluation of the relationship between the rate of urinary excretion and the total elimination rate during the test period. (Table I.) Fig. 3 illustrates the experimental findings in two typical examples. The patients with cirrhosis were arbitrarily divided into 3 groups on the basis of the total elimination rate.

It is seen from the table that the urinary excretion rate during the test period decreases with decreasing total elimination rate, and in all 4 groups

Table I. Urinary excretion of galactose after a single injection of about 500 mg/kg body weight in subjects with normal liver function and in patients with cirrhosis of the liver. The latter are grouped on the basis of the total elimination rate of galactose during the test period

	Amount of galactose injected	Time of plasma concentration = 400 mg/l	Collection interval of urine	Total elimination rate during test period	Average urinary excretion rate in collection interval	Excretion rate in per cent of total elimination rate	Mid-concentration of plasma in urine collection interval	Average urinary clearance in urine collection interval	Total urinary excretion	Total excretion in per cent of amount injected
	g	min	min	mg/min	mg/min	%	mg/l	ml/min	mg	%
A. Normal subjects (N = 9).										
average	32.2	47	18— 46	572	53	9.4	1,100	48	3,820	11.7
stand. dev. ..	6.8	8	3 7	115	17	2.6	230	14	1,130	1.7
range: min.	23.5	37	14 40	406	27	4.3	670	26	2,660	9.2
range: max.	43.0	56	22 55	732	79	14.4	1.530	74	5,330	15.2
B. Cirrhotics, total elimination rate > 300 mg/min (N = 11).										
average	29.4	67	20— 54	356	42	11.6	1.140	35	3,090	10.5
stand. dev. ..	5.2	9	6 15	45	20	5.2	210	12	940	3.9
range: min.	24.6	58	16 41	304	17	5.3	840	20	1,490	4.9
range: max.	40.7	82	23 73	456	74	20.3	1,500	52	4,300	14.8
C. Cirrhotics, total elimination rate < 300, > 210 mg/min (N = 10).										
average	32.0	102	20— 85	246	27	11.2	1,120	23	3,080	10.1
stand. dev. ..	6.1	15	3 12	29	13	6.3	130	10	1,570	6.2
range: min.	26.5	69	16 72	215	10	3.8	890	9	1,290	3.7
range: max.	46.8	128	24 115	298	52	24.1	1,309	44	6,660	25.1
D. Cirrhotics, total elimination rate < 210 mg/min (N = 11).										
average	29.5	120	20— 89	188	23	11.9	1,100	21	2,310	8.3
stand. dev. ..	3.8	26	2 30	19	11	5.2	150	11	930	3.6
range: min.	25.0	91	17 71	156	3	1.7	835	2	930	2.5
range: max.	37.6	179	24 178	209	43	20.7	1,389	41	3,910	15.2

averages about 10 per cent of the latter. As the mid-concentrations of the groups in the sampling interval are almost identical, the values of renal clearance of galactose also show a decrease roughly parallel to the fall in total elimination rate.

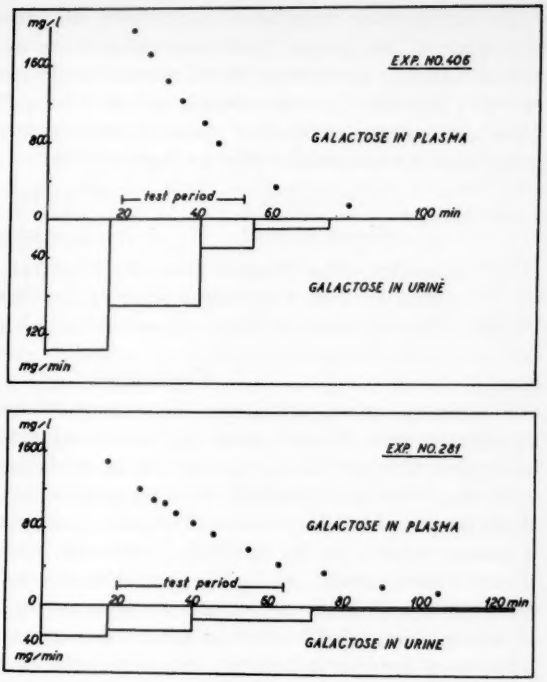

Fig. 3. Plasma galactose curve and urinary excretion in a subject with normal liver function and in a patient with cirrhosis (belonging to group C of Table I) after a single intravenous injection. The amount of galactose excreted in the second and third urine sample was used for calculation of the average excretion rate during the test period.

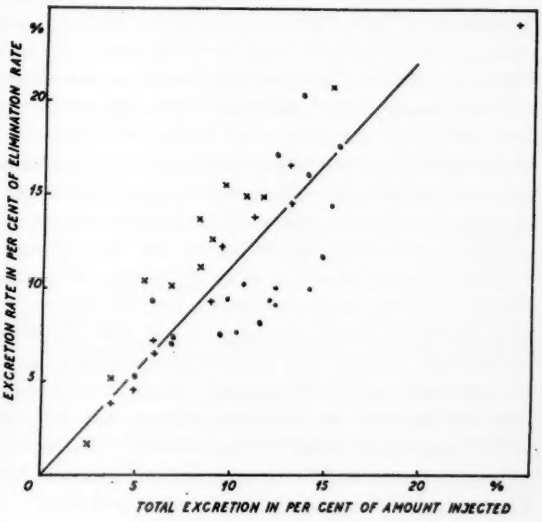

Fig. 4. Correlation between the average urinary excretion rate during the collection interval in per cent of the total elimination rate during the test period and the total urinary excretion in per cent of the amount injected.

• = subjects with normal liver function,

▫ = patients with cirrhosis and total elimination rate greater than 300 mg/min,

+ = patients with cirrhosis and total elimination rate between 300 and 210 mg/min,

× = patients with cirrhosis and total elimination rate smaller than 210 mg/min. The line shows the average relationship between the values in all the patients.

The total amount of galactose recovered in the urine is of the same order of magnitude in all groups. The ratio between the amount excreted and the amount injected is found to be correlated to the ratio between the urinary excretion rate and the total elimination rate during the test period. This correlation is depicted in figure 4, the correlation coefficient (r) is + 0.80. More specifically the correlation may be expressed as

$$\frac{\text{total excretion}}{\text{amount injected}} \times 1.1 = \frac{\text{urinary excretion rate}}{\text{total elimination rate.}}$$

This means that the average urinary excretion rate during the test period may be computed from the total excretion, *i. e.* without catheterisation of the bladder. The standard deviation of the factor 1.1 is 0.3.

Discussion

The data presented agree with the concept of reabsorption of galactose in the renal tubules (GAMMELTOFT and KJERULF-JENSEN 1943) with a low and incomplete threshold at a concentration in the body of 100 to 200 mg/l. The relatively slow rise in clearance at higher concentrations indicates that the T_m of the process is very high, unless the reabsorption at these high concentrations is mainly passive, *i. e.* by diffusion. DOMINGUEZ and POMERENE (1944) found that the galactosuria was highly dependent on the rate of urine flow; our data indicate that this is the case to some extent, while GAMMELTOFT and KJERULF-JENSEN (1943) were unable to demonstrate any such relationship. The reason for this discrepancy may be sought in species differences, in different procedures used for production of diuresis, and in different plasma concentration levels studied. Recently WALDSTEIN *et al.* (1960) found that renal galactose clearances of the same order of magnitude as the glomerular filtration rate may be obtained during water diuresis.

In the usual peroral galactose tolerance tests in which the amount of galactose excreted in the urine is the parameter observed, an abnormal renal handling of galactose may be an important source of false interpretations. In intravenous tests based on the disappearance of galactose from the blood, changes in urinary excretion will only moderately influence the result, the degree of influence somewhat depending on the type of test used.

If galactose is infused at rates lower than the maximal galactose elimination rate of the liver, as for determination of galactose blood clearance (TYGSTRUP and WINKLER 1958), the urinary loss may be disregarded. When larger amounts are infused, the plasma concentration, and thereby the urinary excretion, rises, and as the hepatic elimination is constant, the relative urinary loss rises as well. At a concentration in the body of for instance 2,000 mg/l it will amount to about 20 per cent of a normal maximal hepatic galactose elimination rate of 500 mg/min. Under these conditions the latter only can be determined if the urinary loss is corrected for.

Technically single injection of galactose is the simplest form of intravenous galactose tolerance test and should be well suited for practical assessment of the liver function in terms of maximal hepatic galactose elimination rate. Calculation of this value from the rectilinear slope of the plasma galactose concentration-time curve and the average urinary excretion rate during the test period requires certain assumptions. Thus it must be assumed that other extrahepatic routes of galactose elimination than renal excretion are negligible, and that the apparent volume of distribution of galactose remains constant during the test period.

Since the rate of urinary loss of galactose decreases with decreasing plasma concentrations, one should expect some deviation from rectilinearity of the plasma galactose concentration-time curve (see appendix). Generally this deviation is not discernible, however, probably because it is hidden by the analytical error, and even when a number of curves are pooled and analyzed (TYGSTRUP and WINKLER 1954) there is only a slight and statistically insignificant deviation. The assumption of a constant excretion rate during the test period, used for the correction of the total elimination rate, only is justified if the excretion rate is small in proportion to the total elimination rate. From this point of view it is fortunate that the renal clearance of galactose generally is decreased in patients with cirrhosis of the liver.

Whether the diminished excretion in patients with cirrhosis is due to diminished filtration rate of galactose or to increased rate of reabsorption cannot be stated, as determination of these parameters have not been included in this study. Reduction of the glomerular filtration rate to about 50 per cent of normal has been demonstrated in patients with decompensated cirrhosis of the liver (ÖNEN 1960), and if tubular reabsorption of galactose is unchanged, this may partly account for the diminished excretion. Furthermore the rate of urine flow in the cirrhotics was on an average slightly smaller than in the normals, a fact which may have enhanced back-diffusion of galactose in the tubules.

The reduced renal clearance of galactose may explain why negative results of peroral galactose tolerance tests, based on the quantity of galactose excreted in the urine, are frequently encountered in patients with cirrhosis of the liver.

Appendix

(In collaboration with Chr. GRAM, M. Sc.)

The galactose plasma concentration-time curve following a single injection may be divided into 3 parts (TYGSTRUP and WINKLER 1954). (Fig. 5.)

If the disappearance of galactose from the plasma during the test period is exclusively determined by hepatic elimination and renal excretion, and if hepatic elimination rate and renal clearance are presumed to be constant in this interval, the curve is described by the equation

$$\frac{dc}{dt} = -\frac{GE}{V} - \frac{Cl \times c}{V} \qquad (1)$$

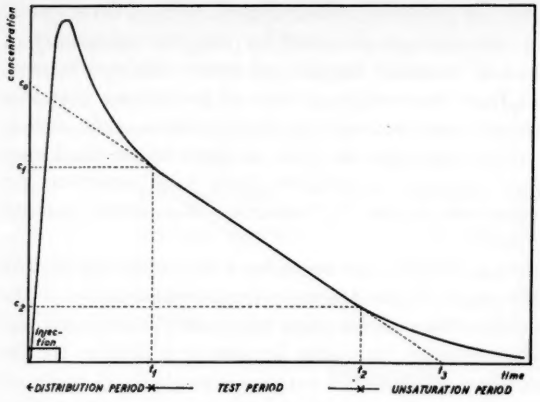

Fig. 5. Theoretical plasma galactose concentration-time curve following a single intravenous injection of 500 mg/kg body weight (TYGSTRUP and WINKLER 1954). The broken lines show the extrapolations and projections used for the calculations.

where c is concentration, t is time, GE is maximal hepatic galactose elimination rate, Cl is urinary clearance, and V is volume of distribution. Therefore

$$c = \left(c' + \frac{GE}{Cl}\right) \times e^{-\frac{Cl}{V} \times (t - t')} - \frac{GE}{Cl} \tag{2}$$

where e is the base of natural logarithmus, and c' and t' are corresponding values of concentration and time, *e. g.* c_1 and t_1 in Fig. 5.

The shape of the curve decribed by equation (2) depends on the relative magnitude of the constants. In order to evaluate the deviation of this curve from rectilinearity in normals and cirrhotics, the curves of Fig. 6 were constructed. The constants used for the special solutions depicted in the figure were obtained by calculating average values of c_1, GE, Cl, and V from the material of group *A* and group *C* in Table I (curve I and curve II, respectively). Curve I therefore may be taken to represent an "average" normal subject, and curve II an "average" patient with cirrhosis of the liver. A number of concentrations were calculated for values of t greater than 20 min and c greater than 400 mg/l, *i. e.* covering the usual test period. These concentrations, with the standard deviation of the analysis, are plotted in Fig. 6. From each series of points the best fitting straight line was computed by regression.

Fig. 6 shows that in both cases examined the deviation of the straight line from the calculated concentrations is smaller than the standard deviation of the analytical procedure. For practical purposes it therefore is warranted to regard the total elimination rate in this period as constant.

Making use of this approximation equation (1) may be written

$$g = -\frac{GE}{V} - \frac{U_2}{(t_2 - t_1) \times V} \tag{3}$$

where g is the slope of the best fitting straight line during the test period, and U_2 is the amount excreted in the urine in this period.

The volume of distribution (V) may be calculated from the concentration after distribution has taken place (c_1) and the amount present in the body at that time (t_1) (*cf.* Fig. 5).

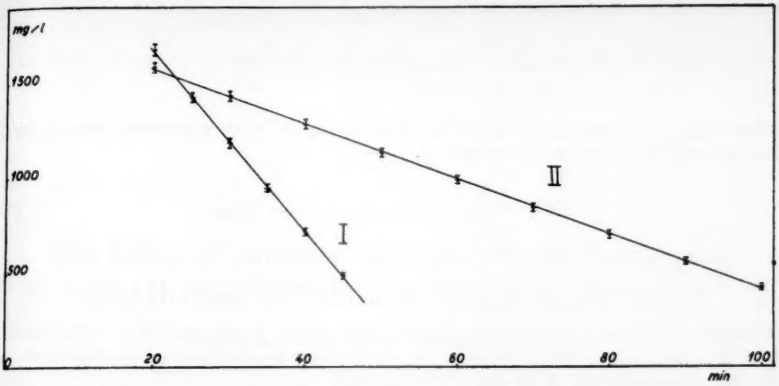

Fig. 6. The influence of the kinetics of urinary excretion of galactose on the plasma concentration-time curve in an "average" normal subject (curve I) and in an "average" patient with cirrhosis (curve II). The concentrations shown (+) were calculated from equation (2), assuming the urinary excretion rate to be proportional to the plasma concentration, and the straight line was calculated by regression from the same concentrations, assuming the urinary excretion rate to be constant according to equation (3). The deviation of the straight line from the calculated concentrations is seen to be smaller than the standard deviation of the analytical procedure, depicted by horizontal bars. (S. D. = 0.01 × c + 10 mg/l.)

$$V = \frac{M - GE \times t_1 - U_1}{c_1} \tag{4}$$

where M is the amount injected, and U_1 is the amount excreted in the urine in the distribution period. Eliminating GE from equation (3) and (4) we get

$$V = \frac{M - \left(U_1 - \frac{U_2}{t_2 - t_1} \times t_1\right)}{c_1 - g \times t_1} \tag{5}$$

where the denominator $c_1 - g \times t_1$ equals c_0 (*cf.* Fig. 5).

From the material of Table I it was calculated that on an average

$$U_1 - \frac{U_2}{t_2 - t_1} \times t_1 = 670 \text{ mg or } 0.02 \times M,$$

and applying this value equation (5) may be written

$$V = \frac{0.98 \times M}{c_0} \tag{6}$$

From equations (3) and (6) it appears that

$$GE = -g \times \frac{0.98 \times M}{c_0} - \frac{U_2}{t_2 - t_1} \tag{7}$$

It was demonstrated (Fig. 4) that on an average

$$\frac{U_2}{t_2 - t_1} \times \frac{c_0}{-g \times M} = 1.1 \times \frac{U_{\text{total}}}{M}$$

where U_{total} is the amount excreted in the urine during the whole experiment, and applying this expression in equation (7) we get

$$GE = \frac{-g}{c_0} \times (0.98 \times M - 1.1 \times U_{\text{total}}) \tag{8}$$

The factor $\frac{-g}{c_0}$ equals $\frac{1}{t_3}$ and t_3 may be determined graphically from the curve. (*cf.* Fig. 5)

If the factors 0.98 and 1.1 in equation (8) are omitted, *GE* on an average will be overestimated by 3 per cent. As this error is unimportant in clinical work, and may be overshadowed by the individual variations of the factors, the equation

$$GE = \frac{M - U_{\text{total}}}{t_3} \tag{9}$$

may be preferred for this purpose.

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Acta physiol. scand. 1961. 51. 275—282

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The Effect of Juvenile Hormone on the Respiratory Metabolism of Silkworm Pupae, as Recorded with a New Semi-Micro Device

By

JOHAN B. STEEN[1]

Received 21 November 1960

Abstract

STEEN, J. B. *The effect of juvenile hormone on the respiratory metabolism of silkworm pupae, as recorded with a new semi-micro device.* Acta physiol.scand. 1961. *51.* 275—282. — The respiration of Polyphemus pupae during normal pupal-adult development has been compared to that during abnormal development as induced by juvenile hormone. Respiration was measured as oxygen-uptake with a new semi-micro recording spirometer. Striking differences were found and it is suggested that the respiratory response may be used as a sensitive assay for the juvenile hormone.

During the time required for an insect to develop from a pupa to an adult, the metabolism, as signalled by the rate of oxygen consumption, increases systematically. This has been documented for several species and orders of insects, particularly in the case of the Cecropia silkworm (SCHNEIDERMAN and WILLIAMS 1953). If such a pupa is injected with juvenile hormone (WILLIAMS 1959), it will not undergo the normal metamorphosis but instead develop into an individual which exhibits a mosaic of juvenile and imaginal characters or even into a second pupa. The object of the present investigation was to compare the respiration of animals following these two paths of development and thereby gain insight into some of the metabolic effects of the juvenile hormone. To this end a semi-micro self compensating respirometer was developed, capable of recording the oxygen consumption continuously over several weeks with an accuracy of some 5 % per 5 ml of gas consumed.

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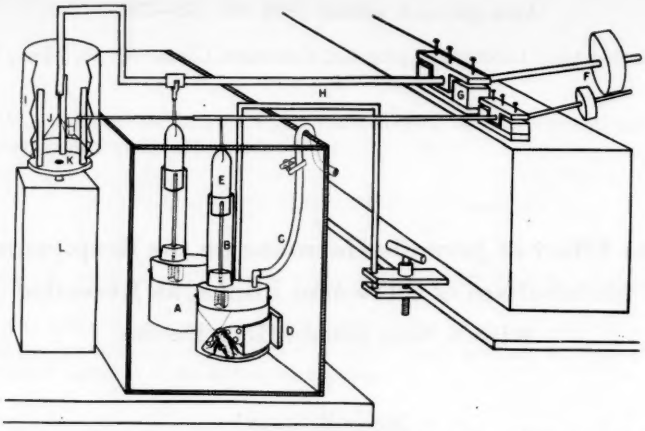

Fig. 1. Device for continuous measurement of oxygen uptake. The total length of the arm from pen to fulcrum is 60 cm, the rest is in proportion to this except for the respiratory chamber which is somewhat enlarged.

A = Respiratory chamber
B = Citric acid jacket
C = Side arm
D = Chamber rack
E = Spirometer
F = Weights
G = Fulcrum
H = Lever arm
I = Drum
J = Tripod
K = Revolving disc
L = Time drive

Materials and Methods

1. *Experimental animals*

The present investigation is based on measurements on a total of 8 female pupae of the giant moth *Antheraea polyphemus*, (a close relative of the Cecropia silkworm) varying in weight from 4.0 to 5.5 g. They had all been stored at 6° C, for about one half year so that they would develop promptly when transferred to room temperature.

2. *Recording spirometer*

This device was designed as a simple and inexpensive recorder for oxygen-uptake in larger insects. The accuracy over short periods of time (two to four hours) is poor, but over longer periods the accuracy is fully sufficient to record constant or systematically changing rates of oxygen-uptake. The device described by BROWN (1957) for the same purpose requires a very accurate temperature and pressure regulation, whereas the device to be described here compensates automatically for external variations in temperature and pressure within 1° C and 15 mm Hg.

a. *Principle*

Two floating respirometers, communicating separately with identical respiratory chambers, govern the position of a pen and a drum respectively. The animal is placed in the chamber connected to the pen. Both chambers contain a carbon dioxide absorbent. The curve drawn by the pen on the drum is, ideally, a record of only the volume change due to the animal since variations in temperature and pressure will influence the position of both pen and drum equally and will not lead to a net movement of the pen on the drum.

b. *Apparatus*

The apparatus consists of respiratory chambers fastened in a thermoregulated water-bath and lever arms each with a fulcrum, a counterweight, a respirometer and a pen or a drum (See Fig. 1). For maximum sensitivity it is essential that the arms and anything attached to them, except the counterweights, be as light as possible.

It is convenient to record from at least two respirometers on the same drum. 300 ml glass jars worked satisfactorily as respiratory chambers for single silkworm pupae. A wet carbon dioxide absorbent N KOH is placed on the bottom and separated from the animal by a non-metallic screen. The chambers are closed off with a two-hole rubber stopper. A section of bent glass tubing is inserted as a side-arm into one hole and into the other hole, a hollow, thick-wall section of Lucite tubing. The latter fits tightly into a second rubber stopper which makes a flexible joint with a section of this glass tubing going up into the respirometer. A section of large diameter glass tubing fits around this stopper forming a jacket. The jacket is filled with a saturated solution of citric acid which has favoruable surface properties and a very low solubility for oxygen[1].

Thin-walled plastic centrifuge tubes (10 to 40 ml) serve as respirometers and are suspended from the light alumium lever arms by wire clips so that they float in the citric acid. The pen fastened at the end of the arm touches the drum as lightly as possible and the counterweight is adjusted so that the lever arms are in equilibrium at any position of the spirometers. The change in buoyancy of the spirometers is compensated by an upward bend of about 8 — 12 degrees in the rod that carries the weight. In order to make the arm more sensitive the compensator arm is bent as shown in Fig. 1 so that the center of gravity of the drum lies on the line through the counterweight and the fulcrum.

The fulcrums are made of wood or aluminium and rest on a hard surface by means of two pointed screws. These tips are on level with the center of the arm. The fulcrums are placed so that the arms are horizontal when the spirometers are half submerged.

The light recording drum is suspended from the arm by a silk thread and a spring wire tripod (Fig. 1 — J), the ends of which are bent slightly outward to fit into holes drilled in the lower end of the drum. The apex of the tripod is in the center of the drum and the feet rest against three vertical glass rods fastened to a clock motor (Gorrell type MG — 1000). Contact at this point is achieved by winding the silk thread a few times before the drum is fitted over the rods. The friction imposed by the glass rods upon the vertical motion of the drum is overcome by the vibration from the motor.

To attain a similar magnification the distance between fulcrum and spirometer is identical on all arms as is the distance from the spirometer to the pen (or the drum).

The recording paper has vertical lines and is changed most easily by first disconnecting the spirometer from the arm so that the latter swings up. After each refilling or change of paper the level of citric acid should still be the same.

c. *Calibration*

The spirometers are calibrated by injecting gas from a syringe through the side-arm while the pen records on a drum which is turned by hand. A scale is made so that it reads the volumes at 0° C. directly. Each ml on this scale occupies $\frac{X \times T}{273}$ cm where **X** is the displacement of the pen caused by the injection of one ml at temperature T. The time calibration is accomplished with the lever arms in a horizontal position. In order to project curves properly onto this base line it is convenient to make a ruler with a straight back edge and a front edge which conforms to the arc traced out by the pen.

[1] Polarographic analysis, sensitive to 10^{-6} M failed to detect any oxygen in saturated citric acid well shaken with oxygen (H. RITTER, personal communication).

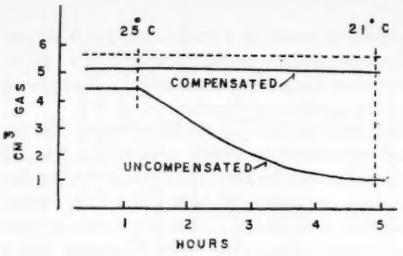

Fig. 2. The two curves illustrate the efficiency of the temperature compensation. They are recorded simultaneously during a temperature fall of 4° C. in the respiratory chamber, but on separate drums. The upper curve is recorded with the device shown in Fig. 1, the lower curve is recorded on a horizontally stationary revolving drum. The dotted line at the top is the base line.

d. *Accuracy of the method*

Gas samples which were withdrawn from the respiratory chambers during experiments and analyzed on the SCHOLANDER 1/2 ml gas analyzer (SCHOLANDER 1947) gave less than 0.03 % CO_2.

The sensitivity can be varied using spirometers with varying diameter. Extremely small spirometers must be avoided, however, since they tend to cling to the tube from the animal chamber. With a spirometer diameter of 1 cm an injection of no more than 0.05 ml of gas can be detected on the drum. This amount of gas is equal to about 0.5 mm vertical movement of the pen.

Variations in the atmospheric pressure of up to 40 to 50 mm Hg are not recorded on the compensated drum; with larger variation the compensation becomes less complete.

Fig. 2 shows recordings during a temperature drop in the waterbath of 4° C. One curve was recorded on the compensated drum, the other on a regular drum. The deflections are 0.1 ml and 3.6 ml respectively. With the waterbath regulated to within 1° C the error introduced by this imperfectness is 0.025 ml.

Repeated gas injections gave an accuracy in the recordings of 10 % for 1 ml samples; 4 % for 5 ml samples and 2 % for 10 ml samples. This uncertainty is due to imperfectness in the balance of the lever arm, to variation in vapor and surface tension and to difficulties in reading the recordings accurately. The accuracy during actual runs is probably no more than 5 % per 5 ml of oxygen consumed. A giant insect pupa uses about 5 ml over 48 hours, which means that values calculated from this on an hourly basis using this method are accurate to about 5 μl. The Warburg manometers are, for comparison, ten times more accurate.

Values for oxygen consumption should be corrected to 760 mm Hg, using the average pressure during the recording time. No humidity correction is needed.

Results

The pupae were anesthetized with CO_2 for 15 to 30 min immediately after they had been removed from the cold room. Six of them received 100 μl of a suspension of juvenile hormone in peanut oil by injection through the mesothoracic tergum just lateral to the midline. Two pupae served as controls, one received 100 μl peanut oil, the other was not injected at all. The small number of controls is sufficient since the normal developmental respiration resembles closely that of the related Cecropia pupa which has been well investigated (SCHNEIDERMAN and WILLIAMS 1953). Seven of the pupae initiated development immediately after they had been removed from the cold room, the peanut oil

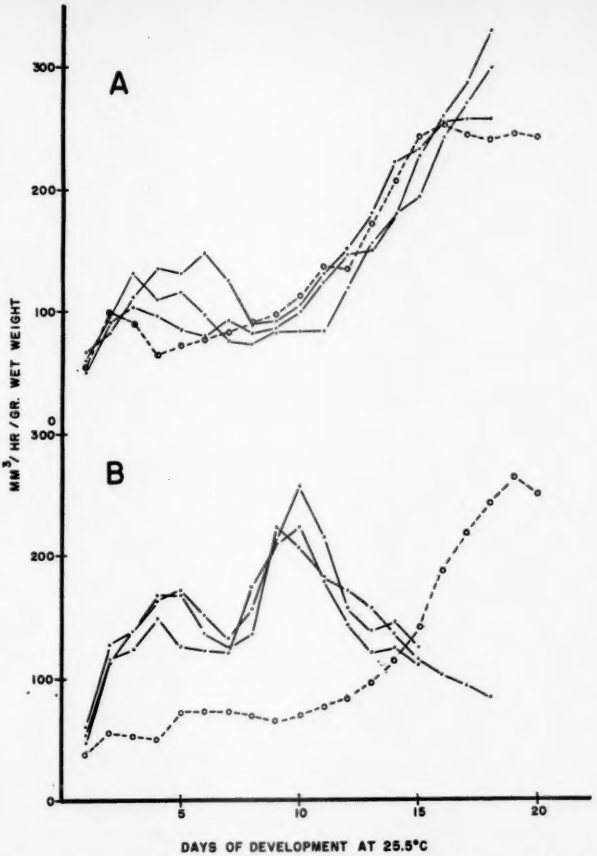

Fig. 3. Rates of oxygen-uptake during development in the Polyphemus silkworms. (*A*) The respiration of three pupae which were injected with juvenile hormone (solid lines) but which developed normally is compared with that of a pupa which was not injected at all (broken line). (*B*) The respiration of three pupae that received injections of juvenile hormone and developed into individuals which had retained several pupal characteristics (solid lines) is compared with that of the pupa which received an injection of pure peanut oil (broken line).

injected pupa started two to three days later. This is well within the range commonly experienced in this species.

Respiratory measurements were started within an hour after injection. The temperature was kept at 25°.5 $\pm$ 0°.5 C, throughout the experiment. When the chambers had been properly assembled and fastened to the clips in the waterbath, they were aerated through the side arm with the disconnected respirometers resting on the citric acid. The side arms were left open to let the

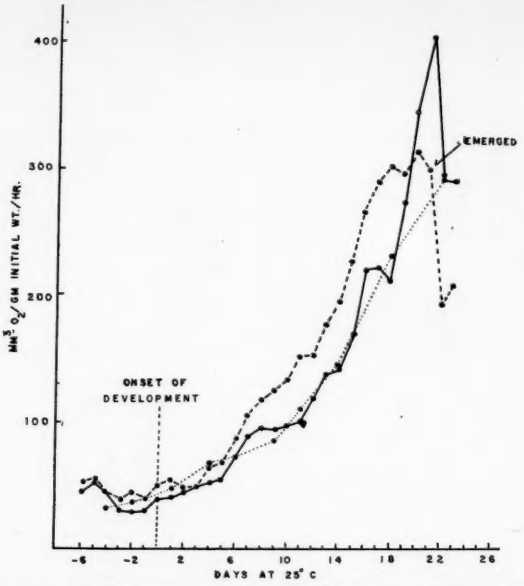

Fig. 4. Rates of oxygen-uptake of three Cecropia pupae during normal pupal-adult development. The dotted curve is taken from Schneiderman and Williams (1953), the two others were obtained with the technique presented above.

spirometers sink and push air out. The side arms were then closed and oxygen bubbled through the citric acid and into the respirometers *via* a J-shaped glass tubing. The recording was interrupted once or twice to renew the carbon dioxide absorbent and to check the stage of development of the pupae. When the animals had finished development, they were scored as to juvenile and imaginal characters according to the morphological bioassay of WILLIAMS (1959).

1. *The oxygen uptake of Polyphemus during normal adult development*

In Fig. 3 A the dotted curve records the characteristic time course in the oxygen consumption of the uninjected Polyphemus pupa during the course of development at 25°.5 C. The shape of the curve is much like that for Cecropia (Fig. 4), except the time is slightly shorter (16 to 17 days as compared to 21 to 22 days). The slight increase in oxygenconsumption during the first few days resembles that in Cecropia and is probably a reaction to the temperature shock the animal experiences when removed from the cold.

Except for the absence of the initial hump, the peanut oil injected pupa showed neither morphological nor metabolic differences compared to the uninjected pupa (Fig. 3 B, dotted curve).

2. *The oxygen uptake of Polyphemus developing in the presence of juvenile hormone*

Despite the fact that the juvenile hormone had been found very potent in earlier tests, only three out of the six injected pupae showed a morphologically abnormal development. The three affected pupae had retained several pupal characteristics at the end of metamorphosis. For example, they displayed pupal cuticle and antennae and they had failed to develop wing muscles. The time-course of oxygen-uptake during this development was very different from the normal picture as demonstrated in Fig. 3-B, although the total uptake of oxygen was not much different. The initial hump is much larger and lasts twice as long as in the normal developing adult and it goes directly over into a second hump which reaches a maximum around the 11th day of development. At this time the morphological development is terminated and the curves fall off gradually returning to the initial level on about the 20th day.

Fig. 3-A (full drawn curves) records the rate of oxygen consumption for the pupae that received active hormone but nevertheless developed into morphologically normal adults. During the first week of development the respiration resembles that of the affected pupae although the initial hump is somewhat lower; during the later part it duplicates that for normal development. This indicates that while the hormone was not active enough to induce morphological irregularities, it was strong enough to induce metabolic deviations.

Discussion

The possibility of using the occurrence of a pronounced initial hump as a bioassay, is suggested. At present a safe judgment cannot be passed as to how dependable this test would be.

A diapausing pupa will normally respond to any injury, even to a needle prick through the cuticle with a temporarily elevated respiration (SCHNEIDERMAN and WILLIAMS 1953). Such an injury metabolism, however, cannot be induced in normally developing animals (HARVEY, personal communication) and it was not found in the pupa injected with pure peanut oil. This does not remove the possibility that the presence of juvenile hormone may increase the sensitivity towards injury or toward a temperature shock under these circumstances.

During the development of the respirometer, I benefited from the expert advice and craftsmanship as well as patience of Mr. R. CHAPMAN. I wish to thank Dr. C. M. WILLIAMS, who kindly supplied both experimental animals and the hormone and who executed the bioassays. I am much indebted to Dr. W. R. HARVEY for his interest and support during all stages of this investigation. The investigation was supported by the Norwegian Research Council and through grants to Drs C. M. WILLIAMS and W. R. HARVEY from the United States Public Health Service.

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Acta physiol. scand. 1961. 51. 283—289

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Conduction Rates of Afferent Fibres to the Anterior Tongue of the Dog

By

J. IRIUCHIJIMA and Y. ZOTTERMAN

Received 23 November 1960

Abstract

IRIUCHIJIMA, J. and Y. ZOTTERMAN. *Conduction rates of afferent fibres to the anterior tongue of the dog.* Acta physiol. scand. 1961. *51.* 283—289. — Rate of conduction and sensory function were studied in single afferent fibres of the chorda tympani and the lingual nerve of the dog. All taste fibres appeared to be medullated fibres (A δ) conducting at rates from 18 to 2 m/sec. The thermal fibres, "cold" as well as the more rare "warm" fibres responding to slight temperature changes (less than 1 °C) were also A δ fibres. All "touch" fibres so far encountered were found to be medullated A fibres. In addition to these medullated fibres some afferent C fibres were found which responded to heavy pressure as well as to extreme temperatures. These fibres obviously serve nociceptive reactions.

Single fibre analysis of mammalian cutaneous nerves has been made by MARUHASHI, MIZUGUCHI and TASAKI (1952) for the myelinated nerve fibres and by IGGO (1959, 1960), IRIUCHIJIMA and ZOTTERMAN (1960) and HENSEL, IGGO and WITT (1960) for the unmyelinated. In these recent papers the specificity of afferent cutaneous C fibres has been established which suggests that, in the cutaneous sensation, a very elaborate, discriminative rôle is played by C fibres.

From the special rôle played by the tongue in taste the fibre spectrum of the chorda tympani and the lingual nerve was suspected to be different from that of the cutaneous nerve. In the present study rate of conduction and sensory function were studied in single afferent fibres of these nerves.

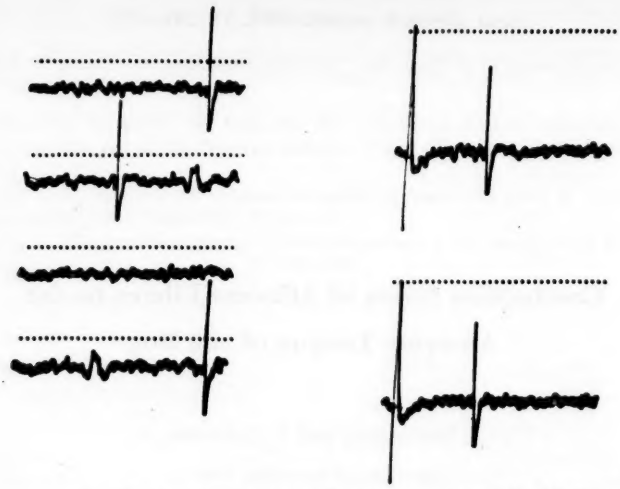

Fig. 1. Determination of conduction velocity. Left: Action potentials of a salt fibre evoked by pouring 0.5M NaCl solution on the tongue. Right: Evoked electrical stimulus applied to the receptive field. Conduction distance: 125 mm. Time in msec. Conduction velocity was determined as 10.4 msec.

Materials and Methods

A total of 16 mongrel dogs of both sexes, weighing between 2.2—11.5 kg, were used in this study. After basal anesthesia with 10 mg Tetroponum and 0.4 mg Scopolamine hydrobromide subcutaneously injected, the animals were anesthetized by i.v. injection of 6 per cent Nebumalnatrium solution containing 1.8 g Pentobarbitone, 4 g Pentobarbitone sodium and 25 g urethane in 10 ml. The initial dose was 0.4 ml of this solution per kg body weight. Additional injections were made to maintain the suitable depth of anesthesia by means of a venous cannula.

After removal of the posterior part of the mandible the chorda tympani was exposed, cut centrally and placed on a black plate in a paraffin pool. The nerve was desheathed and split into such thin strands that even electrical response of C fibres could be recorded. The experiment on the lingual nerve was also made in the same way on the peripheral part of the nerve, below the point where the chorda tympani joins the lingual nerve. The compound action potential was recorded by placing leads on the chorda tympani in its extra tympanic course as well as on its tympanic part in the middle ear. The stimulating electrodes were placed on the peripheral part of the lingual nerve close to its entrance into the tongue at a safe distance from the submaxillary chorda tympani. Records were also made when stimulating this latter nerve. For the sake of comparison, leads were also placed on the central part of the lingual nerve above the entrance of the chorda in order to measure the speed of the fastest A fibres of the trigeminal system.

For taste stimulation, water, 0.5M NaCl, 0.5M sucrose, 0.01M quinine hydrochloride solution, and 0.2M acetic acid were poured on the tongue. The last mentioned three substances were dissolved in Ringer solution to avoid the interference of the water response. Whenever a strand was found to contain a fibre responding to any of the above stated test solutions, the receptive field was determined with a small cotton ball soaked with the solution to which the fibre was responding. In search for the thermosensitive

Tahle I. Conduction velocities of the afferent fibres in the chorda tympani and the lingual nerve of the dog (in m/sec)

Salt fibres	Sweet fibres	Bitter fibres	Acid fibres[1]	Water fibres
1.9	2.0	1.6	6.2	3.5
6.2	4.5	2.8	9.0	3.7
8.7	4.8	3.6	10.0	6.8
10.0	5.3	4.7	12.8	7.3
10.4	12.2		15.3	8.3
13.0	13.7		16.3	9.5
13.0	15.2			10.9
14.4				17.8
14.5				

Warm fibres	Cold fibres	Touch fibres		Pressure fibres	
1.9	Ling. n.	2.9	Ling. n.	0.7	Ling. n.
12.0	3.2	6.5	4.1	0.8	0.4
13.3	7.7	7.1	4.7	0.9	0.5
	8.9	7.5	7.0	1.7	0.7
	10.0	8.3	[2]13.2	1.8	0.7
	10.8	12.5	[2]15.5	2.0	0.8
	11.0	15.0	[2]25.0	4.8	0.8
	11.3	16.4		5.4	0.9
	11.4	24.0		6.2	5.6
				6.5	7.0
				6.8	16.2
				6.9	
				7.4	
				8.2	

[1] Some responded also to salt. [2] Responded also to cooling

fibres a conventional thermode was used and the receptive field was localized by a warmed or cooled metal rod. For mechanical stimulation the tongue was touched with the tip of a brush or pressed with the rounded tip of a glass rod. If the receptive field was determined accurately, it was always possible to stimulate it electrically for the measurement of conduction velocity at such a weak intensity as did not result in the movement of the tongue. The identity of the electrically evoked spike was ascertained by the identical height and configuration of the spikes produced by the natural stimulation using the same speed of recording, as will be seen from Fig. 1.

Results

Taste fibres. Dissection of taste fibres was performed exclusively in the chorda tympani. As is shown in Table I, where all the fibres on which the measurement of conduction velocity was made are found, most taste fibres fell in the category

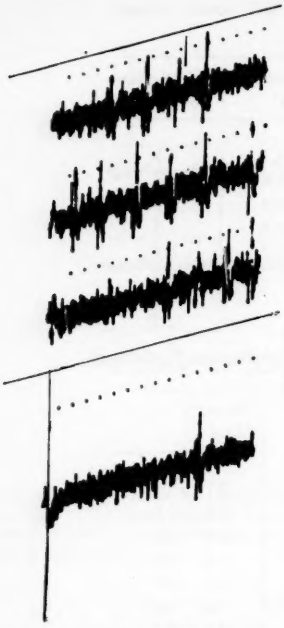

Fig. 2. Afferent C fibre in the lingual nerve. Upper: Response to strong pressure with a glass rod. Lower: Electrically evoked action potential. Conduction distance: 80 mm. Time in 100 msec. Conduction velocity: 0.8 m/sec.

Fig. 3. Compound action potentials;
A. Record from the chorda in the middle ear; stimulating electrodes on the lingual nerve as it enters into the tongue; conduction distance 62 mm. Time in msec; fastest fibres 30 m/sec.
B. Same preparation; leads on the lingual nerve stem centrally of the chorda tympani; conduction distance 57 mm. Time in msec. Conduction rate of fastest fibres 80 m/sec.
C. Same preparation chorda tympani, C elevation. Time in 10 msec. Conduction distance 62 mm. Speed of conduction 1.5—0.8 m/sec.
D. Recording leads on the chorda tympani; stimulating electrodes on the sub-maxillary chorda tympani. Conduction distance 18 mm. Time in 10 m/sec. Rate of conduction 11—4 m/sec.

of the A δ group from conduction velocity. Some of them presented conduction velocities lower than 2 m/sec, which is generally assumed to be the upper limit of C fibres. Nevertheless we hesitate to regard them as C fibres, because as the tongue was stretched and exposed to room temperature, slower rates might be obtained than under normal conditions, though care was taken to keep the rectal temperature of the animal as well as the paraffin pool at around 38.5° C.

Among the taste fibres, the fibres responding to quinine solution presented especially slow conduction rates as compared with the other kinds of taste fibres. This observation is in good agreement with a previous observation (ANDERSSON et al. 1949) that the response to bitter substances is generally built up by smaller spikes, which was also confirmed in this study. Taste fibres did not respond to the pressures which excited the "pressure" fibres.

Thermal fibres. Determination of conduction rate was possible only for three warm fibres in the chorda tympani. Some more warm fibres responding to heating the tongue were observed but were lost when we tried further splitting

for better identification. Of the three fibres, which responded to slight temperature changes (less than 1° C), two conducted at rather rapid rates and presented action potentials of relatively large amplitudes. On the other hand, the conduction rate of the third one was very slow (1.9 m/sec) and the action potential was also relatively small in amplitude. However, from the same reasons as stated before, this fibre probably did not belong to C fibre group.

Cold fibres were found abundantly in the lingual nerve. Their rate varied from 3.2—11.4 m/sec. Those which are listed under the item of "cold" fibre in Table I showed highly sensitive responses to cooling (less than 1° C) and did not respond to touch or pressure. Some fibres which responded to cooling were found to be sensitive also to touch and are listed under the item of "touch" fibre with a special note.

Touch and pressure fibres. In this study the fibres responding to light touch with the tip of a brush are referred to as "touch" fibres and those which could be stimulated only by strong pressure with a glass rod and not by the brush were referred to as "pressure" fibres. All "touch" fibres conducted at the rate of the A class fibres no matter whether they were found in the chorda tympani or in the lingual nerve. As stated above, some "touch" fibres also responded to moderate cooling.

In addition to these A class fibres we found 10 high threshold mechanoceptive fibres, 3 in the chorda tympani and 7 in the lingual nerve, conducting at rates slower than 1 m/sec. The response to strong pressure with a glass rod of one of this kind of fibres is presented in Fig. 2 as well as the action potential evoked by an electrical shock applied to the receptive field for determining the conduction velocity. These fibres also responded to extreme warmth and cold, over 45° C and below 20° C starting from 37° C, just as the cutaneous pressure C fibres did (cf. Fig. 10 of the authors' preceding paper). Some of them, found and localized by applying pressure on the tongue, did not respond to extreme temperature changes on the upper surface of the tongue. However, when the thermode was placed on the lower surface of the tongue, the response to extreme warming and cooling was easily obtained, indicating that these fibres were supplying the lower surface of the tongue.

Typical records of the compound action potential of the chorda tympani will be seen in Fig. 3. The fastest fibres were found to conduct at rates of about 30 m/sec while the fastest trigeminal fibres of the lingual nerve were found to conduct at rates of about 80 m/sec. A C-wave was invariably recorded conducting at rates of 0.8 to 1.5 m/sec when the distal part of the lingual nerve was stimulated. Stimulation of the submaxillary chorda tympani yielded one wave only conducting at rates of 4 to 11 m/sec (Fig. 3 D).

Discussion

According to FOOLEY (1945) 18 per cent of the afferent fibres of the chorda tympani of the cat are unmyelinated while in the dog they constitute 23 per

cent. In comparison with cutaneous nerves the chorda tympani thus contains relatively very few C fibres. Our finding of only 3 single fibres in the chorda conducting at rates below 1 m/sec out of a total of 66 examined chorda fibres seems thus to be in accordance with what could be expected. Besides these 10 fibres which undoubtedly were afferent C fibres we found altogether 6 afferent fibres conducting at rates below 2 m/sec and additionally 5 fibres conducting at rates of between 2 and 4 m/sec. It is very likely that these fibres are of small diameters around or below 1.5 μ with rather underdeveloped sheaths and therefore might have been counted as unmyelinated by the histologists. The finding that the fastest afferent fibres of the chorda tympani conducted at rates of about 30 m/sec is in accordance with the histological finding that the diameters of the medullated fibres of the chorda tympani in the dog do not exceed 6 μ in diameter (ANDERSSON et al. 1950) but is considerably lower than the rates around 50 m/sec found by COHEN et al. (1957) for the fastest fibres of the chorda tympani of the cat. The conduction rates of the B wave, 4 to 11 m/sec, elicited by stimulating the preganglionic secretory fibres running to the submaxillary gland, however, agreed very well with the values of 4 to 12 m/sec found by these authors in the cat.

Stimulation of the submaxillary branch of the chorda tympani nerve gave no C wave which was invariably recorded from the central part of the chorda tympani when the lingual nerve was stimulated. This finding is in accordance also with Foley's finding (l. c.) that removal of the superior cervical ganglion does not result in any diminution in the unmyelinated axons in the chorda tympani of the cat. Thus the C wave recorded by us from the central part of the chorda tympani must be due to the activity of afferent C fibres only.

The largest fibres of the chorda tympani are obviously mechanoceptive. The great number of fibres conducting at rates from 2 to 10 m/sec found in the chorda may to a certain extent depend upon that we used young puppies. But the compound action potential of the chorda gave much the same rates of conduction for the A elevations, as well as for the C wave, in the adult dog. Thus some of the quite small medullated fibres may loose their myelin sheath earlier in their peripheral course and should thus render rather low rates when stimulated from the surface of the tongue.

GASSER (1960) has recently drawn attention to the fact that the speed of the delta elevation comes out to be slower than the rate computed from their diameters, based on the assumption of a linear relationship between velocity of conduction and fibre diameter. It is obvious that the chorda tympani containing relatively so many more medullated fibres of very low conduction speed than cutaneous nerves in this respect seems to be related to the visceral nerves such as the vagus, which in the cat contains thoracic afferent fibres conducting at rates of 2 to 10 m/sec (PAINTAL 1953). Both in the cutaneous and the visceral nerves this elevation has been attributed to A δ fibres.

Only three "warm" fibres were encountered in the chorda tympani. Two

of them were obviously medullated fibres conducting at about the same average rate as that of the "cold" fibres of the lingual nerve. One "warm" fibre conducted, however, at a rate as low as 1.9 m/sec. Contrary to our recent finding of numerous "specific cold" C fibres in cutaneous nerves (IRIUCHIJIMA and ZOTTERMAN 1960) we have not found so far any "cold" fibres conducting at C rates in the lingual nerve. The afferent C fibres which we have been able to trace hitherto seem all to be high-threshold mechanoceptive fibres which to a great extent also respond when their endings are exposed to extreme temperatures.

The taste fibres display a great variety of conduction rates. The highest rate 17.8 m/sec determined for a "water" fibre while for the "bitter" fibres (only 4 fibres tested) the velocities were found to be below 5 m/sec. We do not claim that our failure so far to find any taste or thermal fibres conducting at rates below 1 m/sec proves that in the tongue these modalities are not served by any C fibres. However, as we have applied the same technique with which we were able to record the activities of specific C fibres in cutaneous nerves, and since in this research we actually recorded the activity of afferent C fibres in the lingual nerve as well as in the chorda tympani, we are inclined to assume that taste as well as thermal sensations from the tongue are mediated principally, if not totally, by medullated fibres of small diameters.

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X-Ray Diffraction Studies on Peripheral Nerve Myelin

By

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Abstract

HÖGLUND, G. and H. RINGERTZ. *X-ray diffraction studies on peripheral nerve myelin.* Acta physiol. scand. 1961. *51.* 290—295. — The dimension of the radial repeating unit was measured on peripheral nerve from 15 species belonging to 4 vertebrate classes. In the fishes the dimension was 161 ± 3 Å and in the birds 182 ± 5 Å. In the mammalian and amphibian species the dimensions were in agreement with earlier work (185 ± 3 Å and 171 ± 3 Å, respectively).

Diffraction patterns from brachial plexus nerves of sheep fetuses were obtained from a gestation age of approximately 90 days. Weak lines indicating a lower number of regularly arranged radial repeating units compared to older fetuses were obtained from fetuses between 90 and 105 days gestation age. The diffraction patterns from fetuses older than approximately 105 days gestation age were similar to those from peripheral nerves of adult sheep.

The first low angle x-ray diffraction studies of nerve myelin were reported by SCHMITT, BEAR and CLARK (1935) and SCHMITT, BEAR and PALMER (1941). They found that the radial repeating unit in nerves from various mammals and from reptiles (turtle) was about 184 Å, and that of amphibians about 171 Å. The relative intensities of the various orders of diffraction lines were, in order of intensity, the second, fourth and third.

Several studies have been performed to determine the structure of the myelin sheath by means of x-ray diffraction (SCHMITT, BEAR and PALMER 1941, FINEAN 1953) and electron microscopy (SJÖSTRAND 1953, FINEAN and FERNANDEZ-MORÁN 1957). On the basis of X-ray diffraction data and electron microscopic

Table I. Dimension of radial repeating unit in various vertebrate species

Species	No.	Size of radial repeating unit	Range
Homo	5	185 ± 3 Å	
Monkey	1	185 ± 3 Å	
Sheep	5	185 ± 3 Å	
Rabbit	1	185 ± 3 Å	
Guinea pig	1	185 ± 3 Å	
Mammals, average		185 Å	
Dove	5	182 ± 3 Å	181–183
Duck	3	181 ± 3 Å	180–182
Hen	5	183 ± 3 Å	182–184
Birds, average		182 Å	
Frog	3	171 ± 3 Å	
Toad	2	171 ± 3 Å	
Amphibians, average		171 Å	
Bream	6	161 ± 3 Å	
Mackerell	5	161 ± 3 Å	
Pike	2	161 ± 3 Å	
Salmon	5	161 ± 3 Å	
Trench	1	161 ± 3 Å	
Fishes, average		161 Å	

findings, FINEAN (1953), and later other authors (ROBERTSON 1958, STOECKENIUS 1959, WOLMAN and HESTRIN-LEMER 1960) have proposed models for the molecular structure of the radial repeating unit of peripheral nerve. From electronmicroscopic observations GEREN (1954) found that the repeating units of the myelin sheath are derived from the Schwann cell surface by an infolding process initiated when the Schwann cell envelopes the axon.

In this study the size of the radial repeating unit was measured on peripheral nerves from various species of fishes and birds, and also on mammalian and amphibian species. In connection with investigations on the prenatal functional development of the nervous system (BERNHARD, KAISER and KOLMODIN 1959) diffraction patterns were also obtained from peripheral nerves of fetal sheep. A preliminary report was given at the X. Scandinavian Congress for Physiology, August 1960 (HÖGLUND and RINGERTZ 1960).

Methods

Apparatus Unfiltered copper radiation from a fine focus x-ray tube (Ehrenberg and Spear) was used for all the exposures. A semi-micro low-angle camera with two permanent collimating lead slits mounted on holders allowing lateral adjustments was built for this purpose. The sample-to-film distance was 60 mm. CEA x-ray film, textur X, was used.

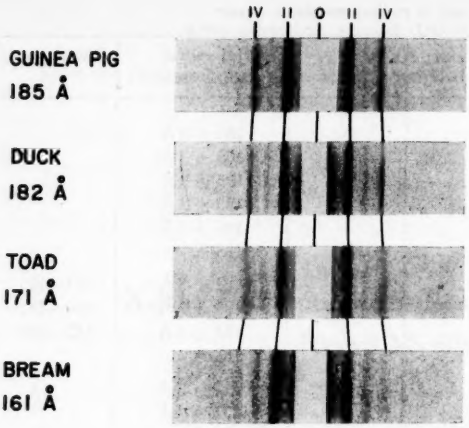

Fig. 1. Low-angle diffracton patterns recorded from peripheral nerves of mammal, bird, amphibian and fish. The centre of the primary beam (0) and the second (11) and fourth (IV) order lines are indicated. The obtained dimension of the radial repeating unit is given to the left.

A one millimeter deep brass chamber providing optimal thickness for diffraction of CuKα radiation by an organic compound was used. The chamber was bounded by two ten microns thick mica windows to provide a moist atmosphere within the chamber during the exposure. For the photometric recordings a Zeiss Schnellphotometer II was used.

Adult nerves The sciatic nerve was used from adult mammals, birds and amphibians and the cervico-brachial plexus from fishes. The animals were killed immediately before the nerve was excised. The human sciatic nerve was taken from fresh autopsy material. The exposure time was 1/2—4 hours. The distances between the obtained lines on the developed film were measured under the microscope. The size of the radial repeating unit was calculated from the lines of the second, third and fourth order. The error of measurement caused by the line width and the air scatter was about 0.05 mm, corresponding to $\pm$ 3 Å.

Fetal nerves Nerves from the brachial plexus of 22 sheep fetuses weighing from 21 to 2,600 g were used. The age of the fetuses varied from approximately 56 days, calculated from the formula by HUGGET and WIDDAS (1951), to maturity at 150 days. The exposure time on nerves from fetuses was 2 to 20 hours.

When nerve material from the same adult animal or fetus was repeatedly examined using varying exposure times, the diffraction pattern was found to be the same if the nerve had not been allowed to dry or exposed to greater changes in the temperature (ELKES and FINEAN 1953, FINEAN 1957).

Results

Radial repeating unit dimension in adult nerve

In Table I are listed the sizes of the radial repeating unit from peripheral nerves of 15 species belonging to four vertebrate classes. In the table the error of measurement, $\pm$ 3 Å, is given after the obtained dimension. Diffraction patterns from species belonging to these classes are shown in Fig. 1.

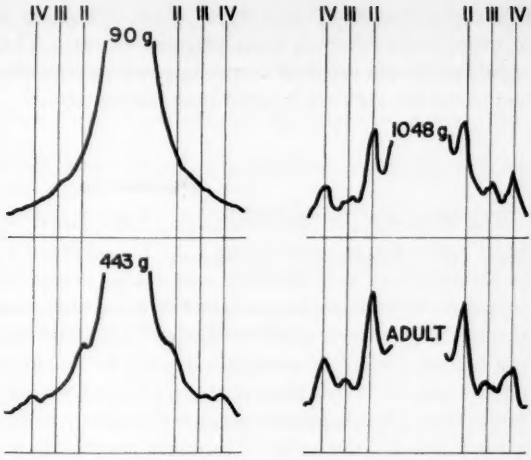

Fig. 2. Photometric recordings of low-angle diffraction patterns from brachial plexus nerves of sheep fetuses.

In the fishes (teleostei) investigated, the radial dimension of the repeating unit was 161 Å, and in the birds 182 Å. The radial dimension in the mammalian peripheral nerve, including that of man, was 185 Å, which is in agreement with the measurements by SCHMITT, BEAR and PALMER (1941) on other mammals. The values for toad and frog, 171 Å, was the same as found earlier. As seen in Table I, mammals, amphibians and fishes showed no variation between different species and animals of the same species. In the birds a slight variation between species and between animals of the same species was found, amounting to $\pm$ 1 Å each.

The relative order of intensity between lines of different order was the same for birds as for mammals and amphibians, namely second, fourth and third, while in the fishes it was found to be second, third and fourth.

X-ray diffraction patterns from fetal sheep nerve

Photometric recordings of diffraction patterns from brachial plexus nerves of fetal sheep of various gestation age are shown in Fig. 2. No diffraction lines were obtained from fetuses smaller than 443 g (gestation age approx. 90 days). The axons were thus surrounded by an insufficient number of repeating units for the recording of diffraction lines.

The diffractograms from fetuses between 443 g and 1,048 g (gestation age approximately 90 to 105 days) showed weak lines indicating a lower number of regularly arranged repeating units compared to older fetuses. In fetuses weighing 1,048 g or more, distinct diffraction lines of the second, third and fourth orders were obtained. The second order lines were the most intense,

followed by the fourth and third. These diffraction patterns were thus similar to those obtained from adult peripheral nerve. The dimension of the radial repeating unit in the fetal nerves from which diffraction patterns were obtained was about the same as in adult mammalian nerve.

Discussion

The differences in the dimension of the radial repeating unit between the birds and mammals was about 3 Å. This difference is smaller than the error of measurement and therefore can not be considered as significant.

At present no data are available showing variations larger than a few Ångström units between different species within the same class of vertebrates but the existence of larger variations can not be excluded. Measurements on fishes, amphibians and birds have yet been reported for only a few species. Variations larger than a few Ångström units are therefore possible particularly within these classes. The relative order of intensity between lines of different order was not the same in the fishes as in the other investigated classes. This may be caused either by the dimension of the radial repeating unit or by a different structural arrangement within the unit.

Diffraction patterns from fetal nerves were obtained from fetuses of approximately 90 days gestation age. It may in this connection be noted that recent electrophysiological studies by BERNHARD, KAISER and KOLMODIN (1959) have shown that the central nervous system of fetal sheep undergoes a rapid functional development during this prenatal period indicating a considerable increase of myelinated pathways.

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A Notice on Clinical Enzyme Unitage

Interpretation and translation of many arbitrary, varied and unrelated clinical enzyme units in current use has been a source of misunderstanding and confusion among clinical chemists. This will continue to be a vexing problem until steps are taken to reach some agreement on a common unitage. The problem is obviously complicated by crude reactants and poorly defined reactions. It is, therefore, manifestly impossible to reach a completely satisfactory solution in every case. However, it was the considered opinion of the Joint Sub-Commission on Clinical Enzyme Units of the International Union of Biochemistry and the International Union of Pure and Applied Chemistry, meeting in Munich in 1959, that some clarification should be attempted, that dimensions should be in basic units, clearly defined and acceptable to all chemists and clinicians. The conclusion and recommendation of the Joint Sub-Commission was that wherever practicable clinical enzyme units should be defined as micro-moles of substrate transformed per minute under specified conditions, and their concentration in terms of a millilitre (or litre) of serum, plasma or urine. Where this is impossible or difficult because of the complex nature of the substrate and uncertainty as to the nature of the products, e. g. with fats, proteins or starch, the units should be expressed in terms of the analysable substances or groups determined to measure the reaction e. g. fatty acid, amino or carboxyl groups and reducing groups. Thus, for instance, with a phosphate the enzyme activity can be expressed as micro-moles of organic phosphate hydrolysed (i. e. of phosphate liberated) per minute per millilitre (or litre); with amylase as micro-moles of reducing group.

This recommendation is in conformance with the report of the Enzyme Commission of the International Union of Biochemistry.

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